THE ENDOCRINOLOGY OF PREGNANCY IN THE MARMOSET MONKEY, CALLITHRIX JACCHUS

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Ph.D.
University of Edinburgh
1981.

DECLARATION

This thesis has been composed by myself, and has not been accepted in any previous applications for a degree or equivalent qualification.

The work, of which this thesis is a record, has been carried out by myself, except where specific acknowledgement has been made. Sources of information have been acknowledged by means of references.

Pamela P. Chambers

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ACKNOWLEDGEMENTS

I would like to thank the Medical Research Council for giving me the opportunity to carry out the research contained in this thesis while in their employment at the Reproductive Biology Unit. I am also very grateful to the staff of the Postgraduate Medical Centre, Raigmore Hospital, Inverness for allowing me the use of their facilities while writing this thesis.

The encouragement of Professor R.V. Short, as my supervisor in the University of Edinburgh, is gratefully acknowledged, as are his criticisms of portions of the manuscript.

The support, advice and criticism of the manuscript by Professor J.P. Hearn, my internal supervisor, has been of constant benefit and I thank him for it. I am also grateful to him for performing the laparotomies and hysterotomies.

My gratitude is extended to many colleagues and friends within the MRC unit. Particular thanks must go to Dr. A.S. McNeilly for his advice and encouragement throughout this study, and for measuring plasma prolactin; to Dr. W.S. O for collaborating on the in vitro culture studies; to Dr. R.K. Kelly and Ms. I Cooper for GCMS measurements of marmoset samples; to Dr. R. Sharpe for his statistical advice and to Mr. S.F. Lunn for his willing assistance in many practical aspects.

Sincere thanks are also due to Mr. Terry Dennett of The Institute of Zoology, The Zoological Society of London,

for the final preparation of the figures, to Mrs L. Northrop for typing the manuscript so meticulously, and to Miss F. Fraser for assisting with the typing.

My thanks go to all the staff involved in maintaining the animals at the MRC Primate Laboratory, Bush Estate. Without their dedicated care, this work would not have been possible.

Finally my thanks go to my family, who have supported and encouraged me throughout this study.

PUBLICATIONS

Chambers P.C. & Hearn J.P. (1979) Peripheral plasma levels of progesterone, oestradiol-17β, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey. Callithrix jacchus. J. Reprod. Fertil. 56, 23-32.

Hearn J.P., Abbott D.H., Chambers P.L., Hodges J.K. & Lunn S.F. (1978) Use of the common marmoset, Callithrix jacchus, in reproductive research, in Marmosets in Experimental Medicine (ed. by Gengozian N. and Dienhardt F.W.) Primates in medicine, 10, 40-49. Karger, Basle.

McNatty K.P., Baird D.T., Bolton A., Chambers P., Corker C. & McLean H. (1976) Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J. Endocrinol. 71, 77-85.

McNeilly A.S., Abbott D., Lunn S.F., Chambers P.L. & Hearn J.P. (1981) Plasma prolaction concentrations during the ovarian cycle and lactation and their relationship to return of fertility post-partum in The common marmoset (Callithrix jacchus). J. Reprod. Fertil. 62,353-360

ABSTRACT

The work in this thesis is a study of the endocrinology of pregnancy in the marmoset monkey, Callithrix jacchus. The hormonal profiles in the peripheral plasma were studied during pregnancy and lactation. Samples were taken from timed pregnancies throughout gestation to investigate ovarian and placental function, the fetal hormonal environment and the fetal development. The hormonal changes and the relative ovarian and placental function during pregnancy in primate species is reviewed.

The marmoset may ovulate and conceive soon after giving birth. Suckling was not associated with any lengthening of the interval between birth and post-partum ovulation despite elevated prolactin levels. Concentrations of LH/CG, androstenedione and testosterone rose in early pregnancy to maximum values at 6-10 weeks. Progesterone, oestradiol-17β and oestrone increased after ovulation and remained high throughout pregnancy. Oestrone was the major oestrogen for the first 12 weeks of pregnancy. Progesterone declined at 3-5 weeks of pregnancy and then remained relatively stable until 12 weeks. At 13 weeks, progesterone and oestradiol rose rapidly above the luteal phase range. Whereas maximum oestradiol and oestrone levels were found during the last week of pregnancy, progesterone levels significantly declined. There was a decline in prolactin levels between 10-19 weeks followed by an increase during the last week of pregnancy.

The corpus luteum was the major source of progesterone and oestrone for the first 50-60 days of pregnancy although its function declined between 20-60 days. In contrast, utero-ovarian oestradiol levels were not related to the presence or absence of the corpus luteum. The placental contribution to hormonal levels became increasingly evident between 50-80 days, and by 90 days, was the major hormonal source and was responsible for the steep increase in progesterone and oestradiol which occurred at this time.

The profile of the placental content of LH/CG, progesterone and oestrogens was similar to the profiles in the peripheral plasma, whereas the profile of the placental content of androgens was different.
In addition, the placenta secretion of progesterone and LH/CG in vitro reflected the proposed in vivo function.

Progesterone, oestrogen and androgen concentrations increased in the fetus between 90-100 days of pregnancy. This was coincident with the increased placental content of all these hormones and the increased progesterone and oestradiol peripheral plasma levels. There was no overall sex difference for any of the fetal hormones. Although there was a sex difference for testosterone and progesterone in male-female co-twins, it appeared that testicular androgen secretion was not a major component in male androgen levels.

The hormonal data indicated that comparable hormonal changes in the peripheral plasma occurred later in gestation than in other species. Also, the ovary remained

important for longer and a major placental influence on hormonal levels occurred later. Prenatal growth measurements showed that marmoset development also occurred more slowly for the first half of pregnancy. However, during the latter half, there were similar patterns and rates of growth as for other primate species, and at birth, a comparable ratio to the adult size was achieved. The data on prenatal growth could be utilised as standards of growth by other investigators as it was related to an accurate gestational age.

CHAPTER 1 : GENERAL INTRODUCTION

This thesis is concerned with the hormonal profiles in the peripheral plasma, and with ovarian and placental function during pregnancy in the marmoset monkey, Callithrix jacchus. The fetal hormonal profiles and development were also assessed in timed pregnancies and related to the hormonal profiles in the peripheral plasma and to ovarian-placental function. The hormones measured in this thesis included progesterone (4-pregnene-3, 20-dione), oestradiol 17-β (1,3,5[10]-oestratriene-3,17β-diol), oestrone (3-hydroxy-1,3,5 [10] -oestratrien-17-4-one), androstenedione (4-androstene-3,17-dione) testosterone (17β-hydroxy-4-androsten-3-one), prolactin and luteinising-hormone/chorionic gonadotrophin (LH/CG).

In this chapter, the quantitative and qualitative hormonal changes in the peripheral plasma and ovarian and feto-placental function during pregnancy are reviewed (section 1.1 to 1.4) for the human and the primate species which have been developed as laboratory models for studies on pregnancy. The review is confined to the aforementioned hormones. In section 1.5, the studies on the marmoset that are relevant to its use as a sub-human primate model in reproductive research are reviewed. The aims and scope of this thesis are given in section 1.6. Relevant references to fetal endocrinology and development are reviewed in Chapters 6 and 7.

1.1 Quantitative changes - Interspecies comparison

One of the first steps in evaluating a species as a possible model for studies on human reproduction is comparison of its hormonal levels and profiles. A quantitative comparison of the values found at the end of pregnancy shows that there is enormous interspecies variation in levels. The highest plasma progesterone and oestradiol values during pregnancy are found in women (Tulchinsky, Hobel, Yeager and Marshall, 1972; Dellertogh, Thomas, Bietlot, Vanderheyden and Ferin, 1975) and marmosets (Hearn and Lunn, 1975; Chambers and Hearn, 1979); intermediate values in the chimpanzee (Reyes, Winter, Faiman and Hobson, 1975) and the lowest values among the Old World primates so far studied: the baboon (Papio hamadryas) (Dawood and Fuchs, 1980; Albrecht and Townsley, 1976), sooty mangabey (Cercocebus atys) (Stabenfeldt and Hendrickx 1973a), African green monkey (Cercopithecus aethiops) (Hess, Hendrickx and Stabenfeldt, 1979), and the macaque species; which include the rhesus (Macaca mulatta), (Neill, Johansson and Knobil, 1969; Bosu, Johansson and Gemzell, 1973a and b; Challis, Davies, Benirschke, Hendrickx and Ryan, 1974; Weiss, Butler, Hotchkiss, Dierschke and Knobil, 1976), bonnet (Macaca radiata) (Stabenfeldt and Hendrickx, 1972), crab-eating (Macaca fascicularis) (Stabenfeldt and Hendrickx, 1973b; Hodgen, Stouffer, Barber and Nixon, 1977) and pig-tailed monkeys (Macaca nemestrina) (Chandrashekar et al, 1980).

Why are there interspecies variations in the hormonal

levels during pregnancy? Factors that could be investigated are production rates, metabolic clearance rates, levels of binding proteins, etc. For example, the substantial difference between the human and rhesus in hormonal levels is due, at least in part, to diminished endocrine activity of the monkey placenta. Associated with the lower progesterone levels in the rhesus are lower placental progesterone concentrations (human: Zander and von Munstermann, 1956; Runnebaum, Runnebaum, Stober and Zander, 1975; Runnebaum, Stober and Zander, 1975; rhesus: Short and Eckstein, 1961; Hagemenas and Kittinger, 1974), a lower progesterone production rate (human: Lin et al, 1972; rhesus: Thau, Lanman and Brinson, 1977) and in addition, lower plasma levels of progesterone binding proteins (human: Slaunwhite and Sandberg, 1959; Doe, Fernandez and Seal, 1964; rhesus: Beamer, Hagemenas and Kittinger, 1972).

Comparison of peripheral concentrations give little indication of the dynamics of the production and metabolism, and does not show whether the availability to target tissues is affected by binding to high-affinity plasma proteins or by the availability of receptors in the target tissue. This point is partly illustrated by a comparison between the human and guinea pig. Both species show a substantial increase in progesterone values to high levels during pregnancy. However, unlike the human, the increase in the guinea pig was primarily caused by a decrease in the metabolic clearance rate whereas in the human the increase

was primarily caused by an increase in the production rate (Challis, Heap, Illingworth, 1971; Illingworth, Heap and Perry, 1970). This alteration in metabolism in the guinea pig was related to the production in pregnancy of a progesterone binding protein, which binds a high percentage of progesterone (Heap and Illingworth, 1974), thus a much smaller amount of progesterone is actually available to the target tissues.

To what extent do the levels achieved by a species during pregnancy reflect the requirement for these levels? It may be of clinical interest to establish the minimum hormonal level compatible with continuing pregnancy so that, if necessary, appropriate therapy could be instituted. Studies of this nature have been mostly confined to the human, but sub-human species may have a significant role to play in assessing the effect of treatments used to prevent abortion, as the treatment may have unexpected effects on aspects of the pregnancy (e.g. fetal development) that were not initially considered (e.g. progestin administration - see Whalen, Peck and Lo Piccolo, 1966).

1.2 The peripheral plasma hormonal profiles during pregnancy

(a) Human

The hormonal profiles are reviewed for specific hormones in the human and are compared with the results for the marmoset obtained in this thesis in Chap 4. The relationship of progesterone, oestradiol and LH/CG to ovarian

and/or placental function are reviewed later in separate sections (1.4 and 1.5).

(i) Progesterone

The conception cycle resembles the non-fertile cycle until 8 to 11 days after the pre-ovulatory LH peak, when hCG is first elevated and progesterone values remain high. Progesterone continues to rise for the first 3 weeks of pregnancy coincident with the rapidly increasing hCG concentrations (Mishell, Thorneycroft, Nagata, Murata and Nakamura, 1973; Corker, Michie, Hobson and Parboosingh, 1976).

It might be expected that the progesterone profiles during pregnancy are well defined for the human, in view of the possible clinical importance in establishing normal levels and trends in the diagnosis and treatment of abortion associated with hormone deficiency. However, there is often disagreement among authors. Between the third and eighth week of pregnancy, some report a further insignificant rise (Tulchinsky and Hobel, 1973), a plateau (Harrison, Youssefnejadian, Brodovcky, Johnson and Dewhurst, 1978) or a fall (Johannson, 1969; Yoshimi, Strott, Marshall and Lipsett, 1969; Mishell et al, 1973; Corker et al, 1976) in progesterone levels. The criteria used for the selection of patients, the sampling regimes and the methods for dating samples must be examined to evaluate the conclusions. For instance, only for 3 of the studies were results calculated using serial samples from several individuals (Tulchinsky and Hobel, 1973; Harrison et al, 1978; Corker et al, 1976). Another

included results from both serial sampling regimes from a small number of patients, and individual samples (Mishell et al, 1973). These studies usually fairly accurately estimated the gestational age by a combination of menstrual history, basal body temperature charts, vaginal smears, ultrasound measurements and/or hormonal measurements around ovulation. In contrast, the stage of gestation in Johannson's study was often estimated on the menstrual history alone and, since several of the patients were scheduled for therapeutic abortion, only limited numbers of serial samples from different times ranges were obtained. The study of Yoshimi et al included data from gonadotrophin-induced pregnancies, in which there may have been over-stimulation of the ovaries (Fogel, Rubin and Ossowski, 1972), and levels in this study were higher than those generally reported by others. The last factor to be considered is the normal individual variation in trends and levels. All of the studies in which serial samples were taken over the whole time range from normal pregnancies showed that there was a wide range in individual values and trends. The diagnostic value, therefore, of following progesterone levels remains debatable, and is possibly only useful if the levels and trends fall well outwith the normal pattern, or at least, are analysed in combination with other data (reviewed: Batzer, 1980).

It is generally agreed that by about the 8-9th week of pregnancy, a steeper increase in progesterone is initiated. Thereafter, there is a sustained rise until 1-3 weeks before term (Johansson, 1969; Lindberg,

Nilsson and Johansson, 1974; Tulchinsky et al, 1972; Batra, Bengtsson, Grundsell. and Sjoberg, 1976) or until term (Yannone, McGardy and Goldfein, 1968; Johansson and Jonasson, 1971). A significant fall in progesterone in the weeks preceding birth has also been reported (Csapo, Knobil, vanderMolen and Wiest, 1971; Turnbull et al, 1974) but other more recent studies have failed to confirm this (loc cit). The decline in progesterone was not observed in all the patients in Turnbull's et al study (1974), and was only reflected in mean values if strict criteria were used in the selection of patients. The fall in progesterone in Csapo's et al study (1971) was small compared with the wide range of individual levels observed at the onset of labour.

The interest in progesterone during the last weeks of pregnancy is partly related to its physiological effects, which have been largely encompassed in the theory of 'progesterone withdrawal' formulated primarily by Csapo and co-workers (1956, 1959, 1977 and others). However, this theory was based primarily on studies in the rabbit, and although a significant decline in plasma progesterone is also observed in other species (e.g. sheep, cow, pig, goat), it does not appear to be a prerequisite for the onset of labour (reviews include: Bedford, Challis, Harrison and Heap, 1972; Thorburn, Challis and Currie, 1977; Challis 1980). The human studies show that there is not a consistent change in the peripheral plasma progesterone level during late pregnancy in women. This has

led to the questioning of the relevance of measuring the peripheral plasma level. It may not necessarily reflect local changes in intrauterine tissues (review: Batra, 1979), the uterine circulation (Thau, Lanman and Brinson, 1976), or the myometrial "action of progesterone" (Csapo, Eskola and Ruttner, 1980). Changes in the binding proteins in the fetal membranes may cause local progesterone withdrawal (Schwarz, Milewich, Johnston, Porter and MacDonald, 1976), which may trigger the initial steps that lead to the preparturient increase in prostaglandin production (Challis, 1980).

(ii) Oestradiol and oestrone

Oestrone and oestradiol rise continuously after the postovulatory nadir (Tulchinsky and Hobel, 1973; Mishell et al, 1972; Thomas, DeHertogh, Pizarro, van Exeter and Ferin, 1973), in part, as a result of the pregnancy-induced increase in sex steroid-binding globulins (Westphal, 1971). Oestradiol has been noted by some authors to be useful for evaluation of early pregnancy. Abnormal oestradiol levels appear to be as predictive as progesterone (see above) or possibly more so (Brown, Evans, Beischer, Campbell and Fortune, 1970; Kunz and Keller, 1976; Jovanovic, Dawood, Landesman and Saxena, 1978; Batzer, 1980).

Unlike progesterone, both oestrogens increase during the first 8-9 weeks of pregnancy but, like progesterone, there is a more rapid increase after the 8-9th week, which continues until term (Tulchinsky et al 1972; Sybulski and Maughan, 1972; Lindberg, Johansson,

Nilsson, 1974; Dehertogh et al, 1975; and others). Turnbull etal (1974) found a sharp increase in the oestradiol levels over the last 6 weeks in the same group of patients for which he reported a significant decline in progesterone (section 1.3a-ii). However, other studies (loc cit), have found no consistent marked change in levels at term.

(iii) Androgens

During pregnancy, there is a significant increase in the testosterone level (Mizuno, Lobotsky, Lloyd, Koboyaski and Murasawa, 1968; Rivarola, Forest and Migeon, 1968; Gandy, 1971; 1977; Dawood and Saxena, 1977; Klinga, Bek and Runnebaum, 1978; Nagami, McDonough, Ellegood, and Mahesh, 1979; Bamman, Coulam and Jiang, 1980) and an increase in androstenedione which may (Mizuono et al, 1968; Gandy, 1971, 1977) or may not (Rivarola et al, 1968) be significant. In those studies in which there was a significant increase in levels, the increase occurred during the first trimester and again at about 37 weeks.

Although there is a sex difference in fetal plasma and amniotic fluid testosterone levels, at least from approximately week 7 to 20 (Chapter 7.1 for references), it was not reported by Gandy (1971, 1977) or by Dawood and Saxena (1977), as being reflected in the maternal plasma. This was felt to be compatible with the view that the effective aromatase system of the placenta would protect the maternal compartment from receiving large amounts of androgens (Beling, 1977). A more recent study

by Klinga et al (1978) did find a significant difference in maternal plasma testosterone levels between 7 and 14 weeks dependent on the sex of the fetus. His study included data from more individuals, and although the difference was significant, the overlap between the 2 groups was large. Therefore determination of maternal testosterone levels would still give no reliable indication of the fetal sex. The other studies either did not cover this period of pregnancy, or had only a limited number of samples to compare.

The significance of raised androgen levels during pregnancy is not known. The increases may simply be related to sex-hormone-binding globulin (SHBG) levels, which increase during pregnancy (Pearlman, Crepy and Murphy, 1967; Rivarola et al, 1967; Anderson, 1974) and following oestrogen administration (Migeon, Rivarola and Forest, 1968). A few investigators have noted that pregnancies with testosterone levels within the normal non-pregnancy range often terminated in abortion. The abnormal conceptus may not have produced enough oestrogen to induce sufficient quantities to SHBG to increase plasma testosterone levels significantly (Vermeulen, Verdonck, Vander Straeten and Orie, 1969; Bamman et al, 1980). The lack of virilisation during pregnancy is often attributed to the lack of increase in the unbound fraction of testosterone (Rivarola et al, 1968; Forest, Ances, Tapper and Migeon, 1971) although Bammann's et al (1980) data indicated an increase in free testosterone level after week 28.

(iv) Human chorionic gonadotrophin (hCG)

It is generally reported, using various assay systems, that hCG is first detected in serum about 9-13 days after ovulation (Vaitukaitis, Braunstein and Ross, 1972; Kosasa, Levesque, Goldstein and Taymor, 1973; Mishell, Nakamura, Barberia and Thorneycroft, 1974; Catt, Dufau and Vaitukaitis, 1975). The detection of hCG-like activity on day 5 by Saxena, Hasan, Haour and Schmidt-Gollwitzer (1974), using a radioreceptor assay, has not been confirmed.

Following its initial detection, hCG rises to maximum values at 6 to 10 weeks of pregnancy (Marshall et al, 1968; Tulchinsky and Hobel, 1973; Braunstein, Rasor, Adler, Danzer and Wade, 1976), and then decreases to approximately 10% of peak values for the remainder of gestation. Some investigators report a small secondary rise in the last trimester, the height of which may be related to the sex of the fetus (Boroditsky, Reyes, Winter and Faiman, 1975; Penny, Olambiwonnu and Frasier, 1976). The actual level measured depends on the assay employed. Biological in vivo and in vitro assay systems generally show peak values 2 to 3 times higher than specific hCG radioimmunassays (Vaitukaitus, 1977).

A correlation between the pattern of hCG and events occurring during pregnancy has led to much research as to its possible functions. For each proposed function, an effect of hCG has been shown, but it has not been verified that the effect is caused exclusively by hCG; and

the role of other factors, including other hormones, has not been excluded.

What are some of the proposed functions? First, the initial detection in plasma corresponds to the implantation period and an immunosuppressive role has been suggested (Adcock et al, 1973; Borland, Loke and Wilson, 1975). Second, rising levels of hCG occur when the life of the corpus luteum is extended into early pregnancy (reviewed: Goebelsmann, 1979; Talwar, 1979). A luteotrophic function exists for only a limited time as corpus luteum function declines despite the continued increase in hCG (see section 1.3). Third, a role in sexual differentiation has been investigated, as maximal peripheral levels correspond to maximal fetal hCG levels (Clements, Reyes, Winter and Faiman, 1976), to elevated testosterone concentrations in testicular tissue (Reyes, Winter and Faiman, 1973) and in fetal serum (Reyes, Boroditsky, Winter and Faiman, 1974), and to the proliferation of Leydig cells in the fetal testis (Pelliniemi and Niemi, 1969; Gondos and Hobel, 1971). Also, hCG will stimulate testosterone secretion from the fetal testis (Abramovich, Baker and Neal, 1974; Ahluwalia, Williams and Verma, 1974; Huhtaniemi, Korenbrot and Jaffe, 1977). Other possible roles of hCG in regulating fetal steroid production (e.g. DHAS secretion by the fetal adrenal) and in its interrelationships with placental steroidogenesis are reviewed in section 1.4.

In contrast to the above suggestions, Gordon and Chard (1977) suggested that "none of the specific

placental proteins has any biological function in the sense of being essential for the mother or the fetus. They are by-products of a more fundamental process concerned with the basic functioning and maintenance of the placenta as an individual and independent organism. In other words, production of placental proteins may indicate nothing other than that the placenta is there". The idea was further put forward that future approaches should perhaps be directed to the study of a combination of hormones and not just further utilisation of purified proteins.

(v) Prolactin

Prolactin in pregnancy is not significantly elevated until 31-33 days following the midcycle LH peak. Its concentration continues to increase approximately linearly until a plateau is reached at the end of pregnancy (Tyson, Hwang, Guyda and Friesen, 1972; Rigg and Yen, 1977; Kletzky, Marrs, Howard, McCormick and Mishell, 1980).

There is much evidence supporting the importance of oestrogens in the physiological regulation of prolactin secretion in man. The initial prolactin increase occurs 1-3 days after the increase in serum oestradiol after implantation (Barberia, Abu-Fadil, Kletzky, Nakamura and Mishell, 1975). Increased levels of oestrogen, either during pregnancy or when induced by exogenous oestrogen administration, is accompanied by a parallel increase in prolactin secretion (Wiedeman, Schwartz and Frantz, 1976; Kletzky et al, 1980). A parallel exists in the increase of oestrogen and prolactin secretion in girls at puberty (Ehara, Yen and Siler, 1975) and their decrease

in ageing women (Vekemans and Robyn, 1975).

The relationship between prolactin and oestrogen is probably not so simple as implied above, at least during pregnancy. The suppression of oestrogen levels during pregnancy, by dexamethasone administration, did not suppress prolactin levels (Kauppila, Puukka and Tuimala, 1979), even though the dosages of dexamethasone used were markedly higher than those which inhibit prolactin secretion in non-pregnant subjects (Cospinschi et al, 1975). During pregnancy other factors, possibly other hormones than oestrogen, prevent the inhibitory action of dexamethasone on prolactin secretion. For example, progesterone also shows a high correlation with baseline prolactin levels and is able to produce small increments in prolactin secretion in vivo (Meites and Clemens, 1972), and progesterone levels are unaffected by dexamethasone administration (Kauppila, Jouppila, Karvonen, Tuimala and Ylikorkala, 1976). In the rat, both oestrogen (Jacobi, Lloyd and Meares, 1977) and progesterone (Caligaris, Astrada and Taleisnik, 1974) induce a significant elevation in prolactin.

(b) Sub-human primates

Non-human primates are widely used as models in research on human reproduction, but detailed hormonal data on the relationships between the sex steroids and chorionic gonadotrophin during pregnancy are available only for the rhesus monkey and, to a more limited extent, the chimpanzee. This section will review the plasma

hormonal patterns found in these two species and in the other primate species in which there have been preliminary studies. Their patterns during pregnancy will be compared to the human. The limitation in the data for some of these species is probably partly due to the difficulty in handling these primates and to the small numbers of pregnant animals available.

(i) Chimpanzee

Among the sub-human primates so far studied, the chimpanzee (Reyes et al, 1975) probably most resembles the human in its peripheral plasma hormonal profiles. Yet the study of Reyes and colleagues is the only report on peripheral plasma hormonal levels, and data was obtained from only 4 pregnant animals, in 2 of which sampling was discontinued well before the end of pregnancy. Of the 2 remaining animals, one produced a twin and one a singleton, therefore the profile and/or level may not be entirely representative of that found in the more usual singleton pregnancies.

Despite these limitations, the data showed that the extension of the life of the corpus luteum into pregnancy was reflected by prolongation of the luteal phase progesterone peak, and subsequently, by a transient decline There was a sustained increase in progesterone initiated sometime after day 80, which is well after the maximum LH/CG values found at day 30-50.

Oestrone and oestradiol rose in early pregnancy, but unlike the human, declined during the first

trimester. Levels subsequently increased steadily and maximum levels were found at term. Prolactin levels also increased during pregnancy, with irregular fluctuations, but there was no analysis of its relationship to progesterone or oestradiol.

Reyes et al (1975) study showed that although there were some differences in the hormonal and temporal relationships from the human during pregnancy, the overall pattern of a sustained increase in levels, over a large proportion of gestation, resembled the human. In addition, the excretion of oestriol during pregnancy (Jirku and Layne, 1965), the metabolism of exogenous oestrone in vivo (Jirku and Lyne, 1965) and the biosynthesis and metabolism of progesterone and oestrogen by placental tissue in vitro (Shinada and Ryan, 1973) suggested comparable feto-placental steroidogenic systems (Ryan and Hopper, 1974).

(ii) Rhesus

The rhesus monkey is the most widely used of the sub-human primate species and several studies have defined in detail the hormonal changes during pregnancy for several hormones.

Comparison of the rhesus monkey with the human shows that the time-course of events surrounding implantation, the initiation of chorionic gonadotrophin secretion, and the subsequent stimulation of progesterone secretion by the corpus luteum in this species (Tullner and Hertz, 1966a; Neill et al, 1969; Meyer, 1972; Reinius, Fritz, and Knobil, 1973; Hodgen, Tullner, Vaitukaitis,

Ward and Ross, 1974; Hodgen, Dufau, Catt and Tullner, 1974; Atkinson et al, 1975) is very similar to that shown in women. However, the post-implantation renaissance of luteal progesterone secretion is clearly distinguishable in the rhesus, whereas it is not in the human, even when daily blood samples are examined (Mishell et al, 1973; Thomas et al, 1973).

Maximum CG values are found earlier in pregnancy in the rhesus than in the human (Atkinson et al, 1975), but in a similar temporal relationship to the progesterone profile and the dispensability of the corpus luteum. Progesterone levels decline from the luteal phase levels during the period of maximum CG levels, followed by a transient increase due to a placental source during the declining phase of CG (Atkinson et al, 1975; see also sections 1.2a and 1.3b). The corpus luteum is dispensable in both human and rhesus (human: Csapo, Pulkkinen, Ruttner, Sauvage, and Wiest, 1972; rhesus: Hodgen and Tullner, 1974; Bosu, Johannson and Gemzell, 1974; (see sect. 1.3a) around the time of maximum CG values. Despite this similarity in these temporal relationships, no causal relationships have been shown. In fact, since CG is widely implicated in stimulating progesterone production from the corpus luteum, much research has gone into confirming and explaining why this function exists for only a limited period of time in both the human and rhesus (human: Hanson, Powell and Stevens, 1971; Runnebaum, Holzmann, Bierwirth and Zander, 1972; Garner and Armstrong, 1977; Rao, Griffin and

Carman, 1977; Halme, Ikonen Rutanen and Seppala, 1978; rhesus: reviewed: Neill and Knobil, 1972; Knobil, 1973).

Probably one of the most striking differences between the rhesus and human in their hormonal profiles is that, in the former, the takeover of hormonal production by the placental tissue does not result in a sustained substantial increase in either progesterone or oestradiol, and progesterone levels usually remain in the luteal phase range (Bosu et al, 1973a,b; Neill et al, 1969; see also section 1.1). The lack of increase in prolactin, except for during the last week of pregnancy (Weiss et al, 1976), may suggest a similar relationship to progesterone and oestradiol as seen in the human (section 1.2a-v) but an acute stimulatory effect of oestradiol on prolactin secretion has not been demonstrated in the rhesus monkey (Butler, Krey, Lu, Peckham and Knobil, 1975; Weiss et al, 1976). The lack of oestriol in rhesus plasma may be due to failure of the rhesus adrenal and liver to produce sufficient amounts of 16-hydroxylated intermediates for oestriol formation by the placenta (Heinrichs and Colas, 1970).

In addition, there are probably significant differences in the metabolism of progesterone and oestradiol during pregnancy between the human and rhesus. Whereas pregnanediol is the major urinary metabolite of progesterone in the human (Bell and Lorraine, 1971), it appears to be androsterone in the rhesus (Plant, James and Michael, 1971; Liskowski and Wolf, 1972). The major oestrogen metabolite is oestriol in the human (Bell and

Lorraine, 1971), but oestrone in the rhesus (Liskowski, Wolf, Chandler and Meyer, 1970; Hodgen et al, 1972).

(iii) Other sub-human primate species

With a view to assessing alternatives to the widely used rhesus monkey, the peripheral plasma levels of several other sub-human primate species have been studied to a limited extent. This has included investigations into several other of the macaque species, such as the bonnet (Stabenfeldt and Hendrickx, 1973; Hodgen, et al, 1977), crab-eating (Hodgen et al, 1972; Stabenfeldt and Hendrickx, 1973), and pig-tailed monkeys (Chandrashekar et al, 1980). The results obtained for peripheral hormonal levels indicate that the rhesus is fairly typical of the macaque species and that in this respect, there is no great advantage to these other macaque species. Another Old World primate, the African green monkey, also showed more qualitative similarities in its progesterone and oestrogen profiles during pregnancy to the macaque species rather than to the human (Hess et al, 1979). Unlike the species mentioned above, the term levels in the baboon are significantly elevated over the luteal phase; however, unlike the human, there does not appear to be any significant change in progesterone levels during the latter half of pregnancy (Albrecht and Townsley, 1976; Dawood and Fuchs, 1980). The sooty mangabey probably most resembles the human with a general upward trend in progesterone during the latter two thirds of pregnancy (Stabenfeldt and Hendrickx, 1973). How suitable this species will turn out to be in other respects as a model for the human has yet to be determined.

1.3 Ovarian function during pregnancy

The major source of progesterone and oestradiol in primates is initially the ovary and then the placenta or feto-placental unit (Heap, Perry and Challis, 1973). This section will review ovarian function during pregnancy and its relationship to the peripheral plasma profiles for progesterone and oestradiol (Sect 1.2) for the human and rhesus. These are the only 2 primate species for which there is much data. The next section will review feto-placental function with respect to progesterone, oestrogen and LH/CG secretion. This review will provide a background for comparisons with the present study in the marmoset (Chapter 5).

(a) Human

The continuance of luteal phase oestradiol and progesterone values during the first 3-4 weeks of pregnancy (section 1.2a) indicates continued or increased corpus luteum function into early pregnancy since, at this stage of pregnancy, ovariectomy will result in a decline in progesterone and oestradiol, and then in abortion (Froewis, 1962; Csapo et al, 1972).

A decline in corpus luteum function after the 4th week of pregnancy, is indicated in the maternal plasma by the decline in 17α-hydroxyprogesterone levels (Yoshimi et al, 1969; Saunders and Elton, 1971; Solomon and Fuchs, 1971; Tulchinsky and Hobel, 1973; Mishell et al, 1973) and the decline or plateau in progesterone levels (section 1.2a). Placental tissue does not possess the 17α-hydroxylase enzyme system at this stage of gestation (Palmar, Blair,

Eriksson and Diczfalusy, 1966) so this hormone is commonly used to reflect corpus luteum function.

During this period of declining corpus luteum function, there is an increasing placental contribution to levels. This was concluded both from lutectomy and removal of the feto-placental unit (leaving the corpus luteum) (Holmdahl, Johansson and Wide, 1971; Csapo and Pulkkinen, 1978, Karim, Rao and Salmon, in press). There appears, therefore, to be a transitional period, during which there is both ovarian and placental contribution to levels. This period corresponds to the period during which peripheral progesterone levels remain stable and oestradiol levels increase gradually. It is not known how much individual variation there is in the proportions of hormone secreted by the ovary or placenta at a specific time during this transitional period, or how critical is the timing of the completion of the luteo-placental shift.

Bilateral ovariectomy experiments have established that the corpus luteum is dispensable after the 7th week of pregnancy. Csapo and Pulkinnan (1978), in a re-examination of the original case reports between 1896 and 1963, found 7 cases (out of 36) of pregnancy maintenance following unilateral ovariectomy prior to the 5th week of pregnancy. The discrepancy in timing can possibly be explained by the 10% incidence of an accessory corpus luteum which is only identified after thorough examination (Csapo et al, 1972). Ovariectomy and replacement therapy experiments have shown that the secretion of progesterone, but not of oestradiol, by the corpus luteum is necessary

for pregnancy maintenance (Csapo, Pulkkinen and Wiest, 1973; Csapo, Pulkkinen and Kaihola, 1973). It should be noted that such experiments do not take into account that the early conceptus may also be capable of steroid hormone secretion which is immeasurable in the peripheral plasma but may be significant physiologically in the uterine environment.

Comparison of utero-ovarian, uterine and peripheral vein hormone levels indicate that the corpus luteum remains active throughout pregnancy (Mikhail and Allen, 1967; LeMaire, Conley, Moffet and Cleveland, 1970; Guraya, 1972; Crisp, Dessouky and Denys, 1973). In addition, morphological studies reveal that the corpus luteum appears functional at term (Green, Garcilazo and Maqueo, 1967; Adams and Hertig, 1969), contains progesterone throughout pregnancy (Zander, Forbes, vonMunstermann and Nehar, 1958), and is capable of synthesizing progesterone in vitro throughout pregnancy (Hammerstein, Rice and Savard, 1964; LeMaire, Rice and Savard, 1968). Compared to the amount of progesterone produced by the placenta at term, the ovarian contribution is small. Its physiological role is obscure but it cannot be vital, for the ovaries can be removed after the first 7 weeks of pregnancy without untoward effect or obvious hormonal changes in the peripheral plasma (loc cit).

(b) Rhesus

The dispensability of the corpus luteum was determined in a similar manner in the rhesus as in the human and it was shown not to be necessary after day 21,

(Tullner and Hertz, 1966b; Bosu and Johansson, 1974; Hodgen and Tullner, 1975; Bosu, Johansson and Gemzell, 1974) and that abortion following ovariectomy could also be prevented by progesterone replacement alone (Meyer, Wolf and Arslan, 1969). The transitory decrease in peripheral oestrogen levels following ovariectomy or lutectomy suggested an ovarian source up to the 5-6th week of pregnancy, but the lack of a decline in peripheral progesterone levels and the persistence of the transitory increase that is observed in normal pregnancies around week 6-7 indicated a predominantly placental source (loc cit).

Although removal of the corpus luteum at known stages of pregnancy can time its dispensability, it does not necessarily establish the functioning of the corpus luteum in normal pregnancies. Comparison of peripheral plasma levels of intact pregnancies with ovariectomised or lutectomised pregnancies may give a limited idea of corpus luteum function in the early stages but it is not useful for later stages. Also, results in the sheep showed that although peripheral progesterone levels in oophorectomised animals were about ½ those in intact animals (Fylling, 1970a), the ovary probably did not contribute 50% to progesterone levels in normal pregnancy as measurement of uterine vein secretion rates showed that the progesterone secretion rate of the placenta was at least 5-fold that of the ovary (Linzell and Heap, 1968).

A better indication of ovarian function during

normal pregnancy is obtained by comparison of hormonal values in the utero-ovarian vein, uterine vein, and maternal peripheral vein. Studies of this nature in the rhesus indicated that the corpus luteum is actively secreting progesterone at day 22 and 157 (gestation length: 165 days), but at day 42, it is either inactive or its secretory activity is very low. It was therefore suggested that a period of low activity or dormancy was followed by a period of renewed function near the end of pregnancy (Treloer, Wolf, and Meyer, 1972; McDonald, Yoshinga, and Greep, 1973; Walsh, Wolf and Meyer, 1974; Walsh, Wolf, Meyer and Robinson, 1979). Walsh et al (1979) further suggested that the renewal of function was limited to a reactivation of progesterone (or progestins) production and that it was relatively inactive with respect to oestrogen production during mid and late pregnancy. This was corroborated by the fact that extensive luteal aromatase activity is found during early pregnancy, but not during late. Likewise, the rise in progesterone output was associated with an increase in the 3β-HSD isomerase activities in the corpus luteum (Sholl, Wolf, and Colas, 1974, 1977). In addition, morphological studies show that the rhesus corpus luteum appears functional at term (Koering, Wolf, and Meyer, 1973; Gulyas, 1974) and that isolated luteal cells from late pregnancy corpus luteum will secrete progesterone in vitro (Stouffer, Nixon and Hodgen, 1979).

Sholl, Anderson, Colas and Wolf (1976) also measured hormonal levels in the utero-ovarian, uterine and peripheral vein and concluded that, at least in some cases,

the corpora lutea remained active throughout gestation and were not quiescent during mid-gestation, and that both progestins and oestrogens were secreted. In addition, in some animals, the corpora lutea did not appear to remain active. Part of the discrepancy between the studies may well lie in the normal interanimal variation in corpora lutea function during pregnancy. This was indicated, but to a more limited extent, in all of the studies if the data from individual animals is examined. The interpretation that oestrogen is secreted throughout gestation by Sholl et al (1976) was based on a greater utero-ovarian vein value than uterine vein value for only 1 of 4 animals at day 160 and the difference was only 100pg/ml. Similarily at day 49, a positive difference for the utero-ovarian vein (400pg/ml) was found for only 1 of 6 animals.

The stimulus for a recrudescence of corpus luteum function has not been ascertained. Factors similar to those operative during the luteal phase of the cycle may be important as luteal cells from late pregnancy exhibit gonadotrophin-sensitive steroidogenesis in vitro similar to late luteal cells from the cycle (Stouffer, Nixon and Hodgen, 1979). However, mCG is either very low or undetectable in both the peripheral and uterine vein blood during late pregnancy in the rhesus (Hodgen et al, 1972; Atkinson et al, 1975; Walsh, Wolf, Meyer, Aubert and Friesen, 1977). Both prolactin and mCG were elevated in late pregnancy in the rhesus (Weiss et al, 1976; Walsh et al, 1977) but undetectable levels following hypophysectomy

and/or fetal death did not result in a lack of progesterone secretion from either the corpus luteum or placenta (Walsh, Meyer, Wolf and Friesen, 1977). This data also indicated that neither the maternal pituitary nor a live fetus were necessary for placental or corpus luteum progesterone production.

It may be that the corpus luteum of late pregnancy responds to different stimuli than that of the cycle. One factor that is consonant with high utero-ovarian vein progesterone levels is the presence of the placenta and fetus as a unit, regardless of whether the fetus was alive or dead. The feto-placental unit may be the source of the luteotrophic stimulus or there may be a feedback relationship between placental progesterone production and corpus luteum function (Lanman, Thau, Sundaram, Brinson and Bonk, 1975).

Identification of a specific obligatory role for the revival of the corpus luteum during late pregnancy remains unresolved. Hormonal patterns associated with the initiation of parturition were similar in ovariectomised and intact animals (Bosu et al, 1974a; and others, see above) and support the concept that the function of the feto-placental unit during late pregnancy is similar, whether or not the corpus luteum is available for revival.

Nevertheless, a functional, though dispensable, corpus luteum during pregnancy may be useful to supplement progesterone production, particularly if placental hormone production becomes marginal or inadequate. This may be of greater significance in a species such as the rhesus in

which placental progesterone production is already low. Such a role for the corpus luteum could be postulated from fetectomy experiments. After fetectomy, maternal peripheral plasma progesterone levels are maintained but the source of progesterone changes from the placenta to the ovary. Peripheral oestrogen levels decline and like normal pregnancy, (Walsh et al, 1979; and others, see above) there is no compensating ovarian production of oestrogen (Tullner and Hodgen, 1974; Lanman et al, 1975). However, in contrast to normal pregnancy, after fetectomy, maternal progesterone levels ultimately decline prior to expulsion of the placenta (Lanman et al, 1975). This presumably reflects declining ovarian progesterone production which may be caused by deterioration of the corpus luteum or of that placental function by which ovarian progesterone production is maintained.

Based on these experiments, Lanman et al (1975) proposed the existence of a feedback control system whereby the placenta stimulates the corpus luteum if progesterone production by the placenta becomes inadequate. The mechanism by which such a feedback control operates is unknown and it may be similar to that which may operate in normal pregnancy (see above). The apparent interanimal variation in corpus luteum function reported for normal pregnancy could possibly be related to interanimal variation in placental function. An obvious feedback control mechanism would be the progesterone level; but it did not appear to be a factor as altering the progesterone levels in both intact and fetectomised monkeys did not affect the

production rate in the pregnant rhesus (Thau, Lanman and Brinson, 1979). However, an effect on the progesterone production rate of a change in progesterone levels at sites less subject to influence by infused progesterone (e.g. fetus, placenta or utero-ovarian circulation) could not be excluded.

Unlike other species so far studied, the life of the corpus luteum of pregnancy in the rhesus is extended into the post partum period as long as suckling continues (Weiss et al, 1973; Stouffer, Bennett, Nixon and Hodgen, 1977). Histochemical techniques and hormonal measurements suggested that human corpora lutea were also functional for a few days after birth but that their activity decreased rapidly (Deane, Lobel and Romney, 1962; Yannone et al, 1968; LeMaire et al, 1971). The biological importance of luteal function during the post partum period may not relate to lactation as ovariectomy during pregnancy does not affect the survival and growth of nursing infants (Tullner and Hertz, 1966; Hodgen and Tullner, 1975). Although the significance of the extension of the corpus luteum life post partum is not known, the presence of a functional corpus luteum may serve to delay the re-initiation of ovulatory menstrual cycles (Goodman, Nixon, Johnson and Hodgen, 1976).

1.4 Feto-placental function

(a) Progesterone and oestrogen

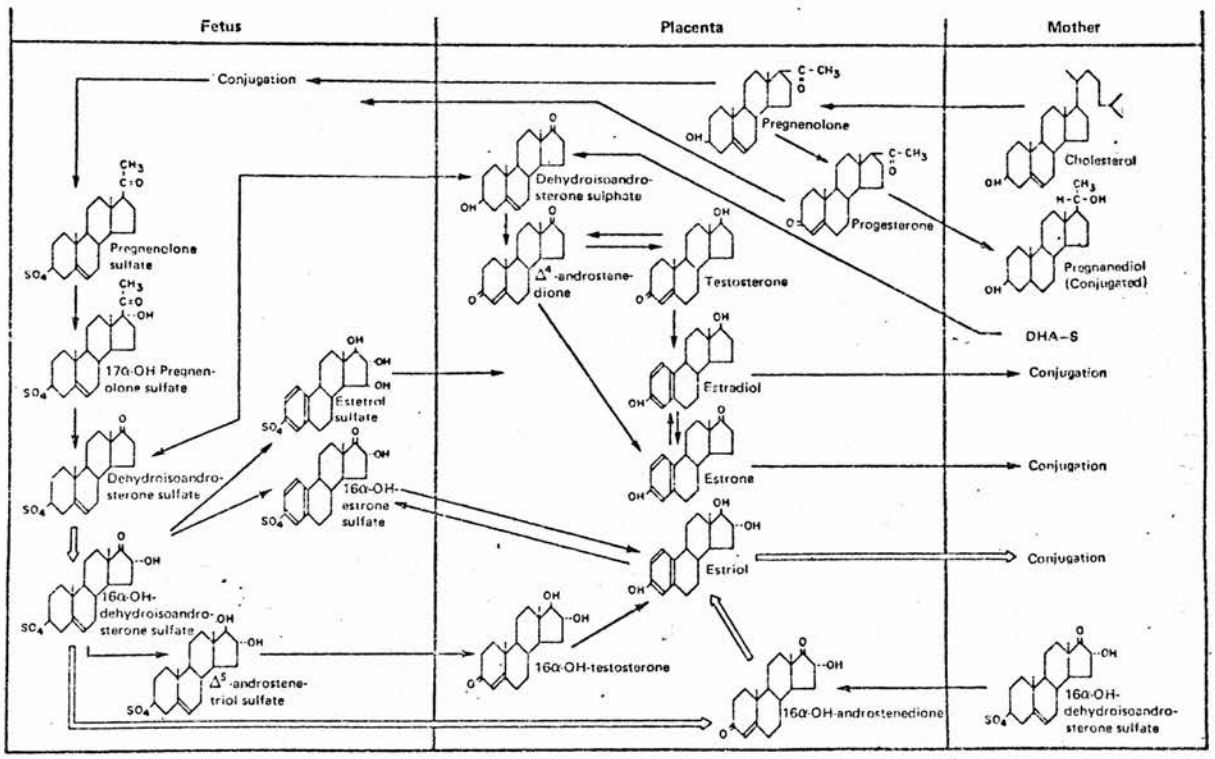
Although the human placenta is capable of maintaining pregnancy after the 7th week (Csapo et al, 1972;

section 1.3a), and there is *in vitro* synthesis of progesterone and oestradiol from endogenous precursors in the placenta from at least 6 weeks (Rao, 1979), the completion of the placental takeover in hormone production in normal pregnancies does not probably occur until closer to 9 weeks Csapo and Pulkkinen, 1978; Holmdahl etal, 1971; Karim etal, in press). This is evident hormonally by the initiation of a steeper increase in progesterone and oestradiol (Tulchinsky *et al*, 1972; DeHertogh, 1975; section 1.2a). Also, the steroid aromatisation capacity of the placenta becomes evident and thereafter increases with advancing gestation (Siiteri and MacDonald, 1966).

Over the last 15 years, the concept has evolved that the placenta is an incomplete steroid-producing organ, unlike the adult adrenal, testis and ovary, and must rely on precursors reaching it from the maternal and fetal compartments. From the unique interdependence of the fetus, placenta and mother, arose the concept of an integrated feto-placental-maternal unit (Diczfalusy, 1969). Several authors have extensively reviewed the biosynthetic pathways of placental progesterone and oestrogen production (Davies and Ryan, 1972; Allen, 1975; Beling, 1977; Klopper and Fuchs, 1977) and Figure 1-1 outlines the major pathways to progesterone and oestrogen formation in the feto-placental unit in the human.

The importance of the fetus for providing androgen precursors for all 3 oestrogens is evident, even though the pathways used for the formation of oestrone and

Fig. 1-1: Progesterone and oestrogen metabolism in the feto placental unit. DHA = deydroepiandrosterone; s = sulfate conjugation. (from Beling, 1977).



oestradiol differ from that for oestriol. Siiteri and MacDonald (1963, 1966) estimated that 90% of the oestriol precursors and 50% of the oestradiol precursors originate in the fetal compartment. The placentae of several primate species also aromatise androgen to oestrogen and none could convert C21 steroids to androgens or oestrogens (human: Bolte et al, 1964a,b,c and d; baboon: Ainsworth, Daenen and Ryan, 1969; Milevich and Axelrod, 1971; chimpanzee: Shinada and Ryan, 1973; orangutan: Ainsworth and Ryan, 1969; rhesus, cynomolgous, squirrel monkeys: Ainsworth et al, 1969; marmoset: Ryan, Benirschke and Smith, 1961). Pregnenolone could be converted to oestrogen in vitro only if the fetal adrenal and placenta were incubated together and not by either tissue alone (iris monkey (Macaca fasciculata): Davies, Ryan and Petro, 1970).

The involvement of the fetus in placental oestrogen production is well documented in vivo in both the human and rhesus, and this fact is used to monitor the well-being of pregnancies with varying degrees of success (reviewed: Klopper, 1977). Fetal death or anencephaly is associated with a decline in the oestrogen content in the urine and/or plasma (human: Frandsen and Stakemann, 1961; Mikakawa, Ikeda, Nakayama and Maeyama, 1976; rhesus: Bosu et al, 1974b; Walsh, Kittinger and Novy, 1979). Oestrogens also decline following fetectomy (human: Kim, Borth, McCleary, Woolever and Young, 1971; rhesus: Tullner and Hodgen, 1974) and dexamethasone administration (human: Ohrlander, Gennser, Batra and Lebech, 1977; rhesus: Bosu

et al, 1974b; Challis, Davies, Benirschke, Hendrickx and Ryan, 1974).

Although a live fetus appears essential for normal maternal oestrogen levels, the picture is complicated by the finding in the rhesus, that fetal anencephaly affects maternal oestradiol but not oestrone. The mechanism by which anencephaly selectively affects maternal oestradiol but not oestrone is not known. One explanation is that there is a major non-placental source of oestrone during late pregnancy. This, however, does not seem likely in view of the fact that oestrone, not oestradiol, is the major oestrogen in the uterine vein both in intact and ovariectomised monkeys in late pregnancy (Dierschke, Wehrenberg, Clark and Robinson, 1978) and that rhesus placental tissue in vitro aromatises C19 steroids with a predominance of oestrone over oestradiol (Ainsworth et al, 1969; Ryan and Hopper, 1974).

In contrast to oestradiol, it is often stated that it is unlikely that any control factor on placental progesterone synthesis originates in the fetus. This is deduced largely from observations that the same experiments of fetal death, fetectomy, suppression of fetal adrenal function or fetal anencephaly have no effect on maternal progesterone levels (loc cit and Allen, 1953; Lurie, Reid and Villee, 1966). It should be noted that most studies measure only peripheral plasma hormone levels and these do not necessarily define the ovarian and placental source of hormones.

There is some evidence which, although not demonstrating a direct fetal role, may suggest that the placenta is not autonomous with respect to progesterone production. Surgically induced intrauterine fetal death in the rhesus eventually produces a marked reduction in maternal plasma concentrations of progesterone (Bosu et al, 1974b) and these authors were probably the first to suggest that the decline in progesterone reflected some undetermined effect caused by the absence of the feto-placental circulation. Lanman et al (1976) later formulated a similar conclusion, based on their observations of a shift to ovarian progesterone production following fetectomy and the abrupt decline in maternal progesterone if ovariectomy was superimposed on fetectomy.

These observations did not show why the placental production of progesterone should decline following fetectomy. It may be due to degenerative changes and modifications in placental perfusion that are associated with fetal removal. Albrecht, Haskins and Pepe (1980) and Albrecht (1980) suggested, based on fetectomy and anti oestrogen (ethamoxytriphetol, MER-25) experiments in the baboon, that the decline in placental progesterone production might be related to the concomitant loss of oestrogen associated with fetectomy. No mechanism of action of oestrogen on sustaining the placental progesterone production was suggested other than that its role appeared essential, rather than stimulatory, in a dose-related fashion. Since MER-25 is a non-steroidal oestrogen antagonist, which interferes with oestrogen binding to its receptor site

(Katzenellenbogen and Ferguson, 1975), it would be desirable to characterise an oestrogen receptor in the placenta. Oestrogen levels are higher in MER-25 treated baboons than in controls, and it would be necessary to determine exactly what is occurring in these treated baboons.

It should be noted that unlike the rhesus, fetectomy in the baboon, resulted in an immediate decline in peripheral progesterone levels. The interspecies difference between the baboon and rhesus may perhaps be due to differences in the placental progesterone-oestradiol relationship (Albrecht et al, 1980), or it may only be that there is no compensating ovarian progesterone production in the baboon as there is in the rhesus. This latter factor was not considered by Albrecht et al (1980), which is somewhat surprising in view of the rhesus results.

A role for the fetus in controlling placental progesterone production was also suggested by the finding in the rhesus that progesterone umbilical vein levels were higher with female fetuses than males (Hagemenas and Kittinger, 1972), and that placental tissue from pregnancies with female fetuses secreted signfic antly more progesterone in vitro than that from pregnancies with male fetuses (Hagemenas and Kittinger, 1974). The influence of the fetal hypothalamic-pituitary axis on placental progesterone biosynthesis was implied by the finding that fetal hypophysectomy significantly lowered the in vitro placental secretion of progesterone (Hagemenas and Kittinger, 1975).

Since addition of pregnenolone abolished the sex difference, it appeared that the step(s) in progesterone synthesis which may be affected by the fetal genotype lie between cholesterol and pregnenolone. The results of these experiments were complicated by the fact that the placental content in these same pregnancies did not always confirm the presence or absence of the sex differences; and it would be desirable to have further validation of the placental culture system to establish whether the endogenous secretion represents primarily preformed or newly synthesised hormone.

Hagemenas and Kittinger (1973) implied that human placental tissue from a pregnancy with a female fetus might also produce more progesterone than from a male fetus. This was not a direct finding, but was deduced from the finding of a sex difference in the fetal metabolism of progesterone. This was based on a total of 7 female and 9 male pregnancies but there was considerable overlap between the 2 groups.

A sex difference in fetal metabolism of progesterone was not confirmed by other authors and there was no indication of a sex difference in the amount of progesterone in the umbilical vein (Effer, Gupta and Younglai, 1973; Tulchinsky and Okada, 1975; Dawood and Helmkamp, 1977). There have not been in vitro studies from the human or any other species comparable to those in the rhesus to confirm a sex influence on placental progesterone secretion.

(b) Chorionic gonadotrophin

The concentration of hCG in placental tissue correlates with the peripheral plasma profile (Diczfalusy, 1953; Hobson, 1971; see section 1.2a). The factors involved in the regulation of hCG production and secretion remain largely unknown although several lines of investigation have been pursued to interrelate its placental production with other physiological factors.

An interrelationship with oestrogen was first shown *in vitro* by cyclic adenoise monophosphate (cAMP), which stimulated the release of hCG with enhanced oestrogen secretion (Hussa, Story and Patillo, 1974; Hussa, Patillo, Ruckert and Scheuermann, 1978). Perfusion experiments with human term placenta showed that hCG increased aromatisation of androgen to oestrogen; presumably by stimulation of cAMP (Cedard, Alsat, Urtasun and Varangot, 1970). hCG may also affect oestrogen production by regulating fetal DHAS production as a precursor for placental conversion to oestrogen (Seron-Ferre, Lawrence and Jaffe, 1978). Not all authors confirm that there is a stimulation of DHAS by hCG *in vitro* (Voutilainen, Kahri and Salmenpara, 1979).

A negative correlation in the peripheral plasma between hCG and oestradiol levels led to the speculation of the existence of a feedback relationship between placental oestrogen production and secretion of hCG (Penny, Parlow and Frasier, 1979). Yuen, Cannon, Lewis, Sy and Wodley (1980) then suggested a role for prolactin in the control of hCG and oestrogen secretion by the feto-placental unit.

This was based on their observations of a significant negative correlation between hCG and prolactin, a positive correlation between prolactin and oestradiol, and that long term suppression of prolactin was associated with augmentation of the hCG peak in the first trimester and in late pregnancy. Their data were quite limited, e.g. augmentation of the hCG peak with long term suppression of prolactin was observed in only 1 patient, and therefore more definitive studies would be necessary to elucidate the relationship.

Recently, some interest has focused on the relationship between placental luteinising hormone-releasing hormone (LHRH), progesterone and hCG. The placenta contains large quantities of biologically active LHRH which is biochemically and immunologically indistinguishable from hypothalamic LHRH (Gibbons, Mitnick and Chieffo, 1975; Siler-Khodr and Khodr, 1978).

LHRH added to placental organ cultures enhanced hCG secretion in a dose-dependent manner. The responsiveness of the 2 placentae cultured was not the same but this may be because of the differing gestational ages of the placentae (Khodr and Siler-Khodr, 1978). If one examines the data, it is difficult to interpret what is actually happening in vitro. The stimulation of hCG by LHRH was greatest on the 1st day, still significant on the 2nd day, but there was no effect by the 3rd and 4th days. Other studies using organ cultures of human placental tissue have suggested that the factors involved in the secretion of

hCG in the media change with successive days of culture, and that it is possibly not until the third day that the secretion is not primarily the release of preformed hormone. (Golander, Barrett, Tyrey, Fletcher and Handwerger, 1978; Huot, Foidart and Stromberg, 1979). At this time (day 3-4), the secretion of hCG begins to increase. It was postulated that there is an inhibitory factor in the placenta present in the explants at the beginning of culture which disappears with time. In Khodr and Siler-Khodr (1978) study, hCG secretion did not increase at the 3rd -4th day of culture but continued to decline with each successive day. However, they did show that there was some *in vitro* synthesis by comparison of the amount secreted *in vitro* with the placental content.

In contrast to the *in vitro* results, *in vivo* administration of LHRH failed to increase maternal hCG levels (Tamado, Akabori, Konuma and Araki, 1976; Seppala Wahlstrom, Lehtovirta, Lee and Leppalouto, 1980). The authors of these papers acknowledge that this may not be surprising since circulating LHRH may not influence placental metabolism, and that a high enough dose of LHRH may not have been used because there are very high con-concentrations of LHRH in the placental tissue and high dosages are required for an effect *in vitro*.

In addition to enhancing hCG production *in vitro* the addition of LHRH to placental cultures inhibited progesterone secretion in a dose-response manner (Wilson and Jawad, 1980). Placenta obtained earlier in gestation (12

and 22 weeks) demonstrated greater spontaneous progesterone production and increased sensitivity to LHRH than term placenta. The dosages of LHRH used were far in excess of those in the hCG stimulation experiements and were possibly pharmacological. Since the weight of the placental tissue cultured is not given, it is not possible to compare the dosage with the reported placental content of LHRH.

The *in vitro* results cited above are difficult to reconcile with the *in vivo* situation because the period of greater sensitivity to LHRH in suppressing progesterone coincides with the period of the establishment of placental production and of increasing placental concentrations of LHRH. Likewise, although LHRH stimulated hCG *in vitro*, the placental concentration of LHRH is still increasing when hCG production is in decline. These observations do not exclude such a relationship but it seems additional factors are involved.

A more direct interrelationship between hCG and progesterone was indicated by the suppression of hCG secretion *in vitro* by progesterone, pregnenolone and 20α-dihydroprogesterone and by the significant suppression of the augmented hCG response to dibutyl cAMP. However, the addition of aminoglutethimide, which blocks the synthesis of progesterone, did not enhance hCG secretion (Wilson, Jawad and Dickson, 1980). This study suggested that the increasing placental synthesis of progestins may be responsible for the decline in hCG secretion, but the mechanism by which progestational steroids, or their metabolites, decrease hCG secretion is not known.

The study of the factors involved in the control and synthesis of placental hormones must be considered to be still in its initial stages. Most of the theories are based primarily on peripheral plasma hormonal interrelationships with generally little experimental evidence for support, and/or on what must be regarded as preliminary in vitro studies. The use of greater numbers at more stages of gestation would benefit most of the studies cited. In addition, far more rigorous investigations are necessary. Organ placental culture may prove to be a suitable method, but it is difficult to delineate the factors controlling the endogenous secretion of hCG in vitro; factors which may well change with successive days in culture and thereby confound experimental results. Although organ culture may be desirable because the tissue is kept basically intact, it also is a highly complex system.

1.5 The marmoset monkey

The macaque monkeys, especially the rhesus macaque, have been most frequently used species in biomedical research. The increasing costs of obtaining and maintaining rhesus monkeys, the increasing demands for laboratory primates, and the differences between the rhesus and the human in their reproductive endocrinology has prompted a search for other suitable species of subhuman primates. As a result, considerable attention has focussed on marmosets. Marmosets have been maintained in captivity for studies in behaviour (reviewed: Rothe, Wolters

and Hearn, 1978), conservation and biology (reviewed: Bridgwater, 1972; Kleiman, 1977) and various aspects of biomedical research, such as immunology, virology, genetics, teratology and endocrinology (reviewed: Gengozian and Dienhart, 1978).

In physical terms, the marmoset has several advantages as a laboratory primate. Their small size makes them relatively easy to handle for routine procedures such as blood sampling, and they are inexpensive to house and feed. The marmoset breeds rapidly in captivity, shows no lactational anoestrus, often produces 2 sets of twins each year and reaches maturity within 2 years (Hearn, Lunn, Burden and Pilcher, 1975; Hearn, Abbott, Chambers, Hodges and Lunn, 1978; Ogden, Wolfe and Dienhardt, 1978; Hampton, Gross and Hampton, 1978). The high fecundity of the marmoset is one of the major attributes that make this species highly suitable for studies in reproductive biology and means that it is one of the few primates that can be maintained in large self-supporting colonies at a reasonable cost.

The disadvantages to using the marmoset are that their small size causes problems if complicated surgery or the withdrawal of large quantities of blood is required. Also, since marmosets do not menstruate, show any externally obvious oestrus, or clearcut cyclical vaginal cytology (Hampton and Hampton, 1975; Hearn and Renfree, 1975), there is no simple procedure by which the stage of the ovarian cycle can be determined. The

simplest method at present of overcoming this problem is by following peripheral plasma progesterone as there is a clear hormonal cycle (Preslock, Hampton, and Hampton, 1973; Hearn and Lunn, 1975).

The studies in this thesis were concerned with the hormonal profiles in the peripheral plasma during lactation and pregnancy, the ovarian and placental function, and the fetal hormonal environment and development. (See Sect. 1.6) To date, the reproductive endocrinology and fetal development of the marmoset has received little detailed attention. Preliminary studies on the endocrinology of the cycle (Preslock et al, 1973; Hearn and Lunn, 1975) and pregnancy (Hearn and Lunn, 1975) indicated that the marmoset may show some quantitative and qualitative similarities to the human in its hormonal profiles in the peripheral plasma. These studies did not cover a wide range of hormones or define the hormonal changes in detail, and the assays used either were not specific (Preslock et al, 1975) or were not fully validated for marmoset samples (Hearn & Lunn, 1975). Identification of the urinary metabolites of progesterone and oestrogen in the pregnant marmoset (Shackleton, 1974; Lunn, 1978) suggested that, also similar to the human, there was a high excretion of the metabolites of these hormones during pregnancy. Whether the reasons for the high hormonal levels during marmoset pregnancy are similar to those in the human is not known, as there have been no studies in the marmoset on the steroid metabolic clearance rates,

the levels of steroid binding proteins in plasma or on ovarian-placental function.

Little is known about ovarian and placental function during pregnancy in this species. Hearn (1978) suggested that the corpus luteum may be removed after approximately the 6th week of gestation without disrupting the course of pregnancy, but this was not considered definitive as the stage of gestation was estimated and interstitial tissue in the ovary of the marmoset undergoes considerable luteinisation, which may be a source of progesterone (Hampton and Taylor, 1971). Immunisation of the marmoset against β-hCG suggested that this hormone may have a role in maintaining the corpus luteum of early pregnancy and/or in implantation, because immunised animals cycle normally, but remain infertile, when antibody titres are high. (Hearn, Short and Lunn, 1975). These experiments also indicated that immunisation against β-hCG may affect ovarian and/or placental function later in pregnancy because when these actively immunised animals returned to breeding, they experience a series of recurrent abortions prior to the 10th-12th week of pregnancy. (Hearn, 1976).

Nothing has yet been reported on the fetal hormonal environment of marmosets and there is little data on prenatal or placental development. Studies have, however, shown that the marmoset is unusual among primates in that it regularly produces dizygotic twins (Hampton et al, 1978; Odgen et al, 1978). There is an early development

of a shared chorionic cavity and shared placental circulation (Hill and Hill, 1932; Wislocki, 1939), and co-twins are XX/XY haemaopoietic chimeras. However, the freemartin condition or masculinisation of the female fetus is not observed in females born co-twin to a male. (Benirschke and Brownhill, 1962; Benirschke, Anderson and Brownhill, 1962; Gengozian, Batson and Eide, 1964; Benirschke and Layton, 1969).

Phillips (1976) studied embryonic development during approximately the first half of pregnancy, and suggested that embryos could be assessed according to the Streeter classification of developmental horizons, and that there may be a lengthening of the early stages of embryonic development. The reason for the lengthening of the early stages of development are not known but may be related to the unusual embryology of the marmoset. Some information is also available on placental (Hampton, 1975), fetal gonadal (Hampton and Taylor; 1971) and adrenal (Benirschke and Richart, 1964) development. However, the data in all of the studies on fetal and placental development was not related to accurately dated pregnancies.

1.6 Aims and scope of this thesis

The endocrinology of pregnancy has been most extensively studied in the human and rhesus, and to a lesser extent in other Old World primate species (See section 1.1-4). In contrast, there has been no compre-

hensive study on the endocrinology of pregnancy in New World primates. The common marmoset, a New World primate, has proved to be a suitable laboratory primate model for studies in reproductive biology and there have been a number of applied studies on aspects of its reproductive biology (Sect. 1.5) which indicated that the marmoset may have considerable inherent scientific interest. First, the marmoset is unusual among primates in that it does not show lactational anoestrus. It may be that suckling is not associated with elevated levels of prolactin or that the marmoset is not susceptible to the inhibitory influences of suckling and/or prolactin on reproductive function. Second, although further removed taxonomically from the human than the much used rhesus monkey, the high hormonal levels during pregnancy in the marmoset appear to resemble the human more than the rhesus, but the relationship between these high hormonal levels and the ovarian-placental secretion of these hormones is not known. Third, there is some indication that, compared to the human, there may be a lengthened period of embryonic development. However, the data obtained was not related to accurately timed pregnancies. Furthermore, the delay may apply only to embryonic development or, in addition, to the hormonal profiles in the maternal and fetal plasma, and to the changeover from ovarian to placental function. Fourth, the embryology of the marmoset is unusual but the effect of this on the fetal hormonal environment in which normal fetal development and sexual differentiation occurs

is not known.

The studies in this thesis were designed to investigate the aforementioned aspects and to provide detailed information throughout pregnancy on the inter-relationships between the peripheral plasma hormonal profiles, ovarian-placental function and fetal endocrinology and development. These aspects were studied in accurately dated pregnancies. This is the first such study for a New World primate and the data obtained allowed comparison of this species with the Old World sub-human primates and the human. The studies in this thesis are described below:

1. The hormonal profiles during lactation and pregnancy were studied. The detailed analysis of the trends and interrelationships of a wide range of hormones throughout gestation and for specified periods are an essential base for the remainder of the studies in this thesis.

2. The changes in ovarian-placental function thoughout pregnancy were assessed and related to the changes in the hormonal levels in the peripheral and fetal plasma. This is the first primate species in which ovarian-placental function has been defined throughout pregnancy at frequent intervals in timed pregnancies.

3. The changes in the fetal hormonal environment throughout pregnancy were investigated and related to the sex of the fetus, to the placental function and to the stage of fetal development.

4. The fetal development was studied in timed pregnancies throughout gestation. It was necessary to confirm, and to more accurately define, the nature of the delay in the prenatal development; and to relate the timing of the hormonal events outlined above (nos. 1-3) to the prenatal development. It was also desirable to produce a set of easily utilised fetal growth standards so that future investigations in this species could be related to an accurate gestational age.

The general procedures applicable to all the studies reported in this thesis are given in Chapter 2, and the development and validations of the radio-immunassays for marmoset samples in Chapter 3. The hormonal profiles in the peripheral plasma during pregnancy and lactation are reported in Chapter 4. Chapter 5 reports on the ovarian and placental function both in vivo and in vitro. Studies on fetal hormones and fetal development are given in Chapters 6 and 7 respectively. In Chapter 8, a brief general discussion of the findings in this thesis in relation to the aspects outlined in this chapter is presented.

CHAPTER 2 : MATERIALS AND METHODS

2.1 Animals

The animals used in this study were common marmoset monkeys, Callithrix jacchus. They were maintained at the MRC Unit of Reproductive Biology Primate Laboratory at the Bush Estate, Midlothian. Animals used were adults (over 2 years old and 280gm body weight) either obtained from the wild, more than 18 months before their use in experiments and fully adapted to captive conditions, or born in captivity. All females involved in the studies on pregnancy had at least one previous successful pregnancy.

2.2 Management

Marmosets were kept in family groups of a male-female pair with their young of less than 300 days of age. Hearn, Lunn, Burden and Pilcher (1975) have published full details of the management of this colony.

2.3 Collection of blood

Blood samples were taken between 09.00 and 12.00 hours without anaesthesia from the femoral vein using an 0.46mm diameter (27 gauge) needle and a heparinised 1ml syringe, and placed immediately on ice. The syringe was sealed with a steriseal cap, centrifuged at 2500 rpm for 20 minutes at 4°C, and the plasma was stored at -20°C until assayed. Animals were either restrained manually by a handler or in a restraining device that allowed a single person to manipulate the monkey and collect the

blood sample (Hearn, 1977).

When either system was in use, the marmosets remained relaxed and appeared to be under little stress. After collecting the blood sample, the animals were given 0.1-0.2ml of iron syrup ("Fersamal", Glaxo Laboratories Ltd.) as a reward, and for replacement of iron. An 0.1-0.3ml intramuscular injection of Imferom (Fisons Ltd.) was also given once weekly to animals undergoing serial bleeds.

2.4 Diagnosis of pregnancy

After parturition or abortion, 35 females were bled every 2-3 days and progesterone concentrations were measured to detect ovulation. Pregnancy was confirmed 3-4 weeks after ovulation by continued luteal phase levels of progesterone and by an increase in the uterine diameter.

A rise in progesterone of more than 10ng/ml which was followed by luteal phase levels (30ng/ml) was considered to be the first day (Day 1) of pregnancy. All pregnancies dated by the progesterone rise will be referred to as timed (T) pregnancies. The interval between the rise in progesterone concentration and the day of birth was used to calculate the gestation length.

Samples were also taken from animals in which the stage of gestation was estimated by abdominal palpation only, in order to supplement the number of samples at particular stages of gestation. Pregnancies which were carried to term were dated retrospectively from the day of

birth. The stage of gestation in these animals will be referred to as estimated (E).

2.5 Surgery

Surgery was performed under aseptic conditions. All animals for surgery were anaesthetised with an intramuscular injection of 0.5-0.8ml "Saffan" (18mg/kg) (Alphaxalone 0.9% w/v, Alphadolone acetate 0.3% w/v; Glaxo Laboratories Ltd.), and received an intramuscular injection of 0.2ml Penidural (Fortified Injection Veterinary - John Wyeth and Brother) and 0.3ml of Imferom. The dose of anaesthetic was sufficient to maintain animals under controlled, deep anaesthesia for a period of about 1 hour. When the anaesthetic had taken effect the animals were shaved in the appropriate region, the skin disinfected with Hibiscrub (I.C.I. Parmaceuticals Ltd.), and the surrounding area covered with surgical dressing.

Prior to surgery, all females were weighed and a 1.0ml blood sample was taken from the femoral vein. Immediately following surgery, females were reweighed and a 0.3ml blood sample was taken.

2.6 Surgical procedures

In the course of this study, the following surgical procedures were used:

1) Laparotomy

2) Hysterotomy

Both these operations were performed by Dr. J.P. Hearn with assistance by the author.

1) Laparotomy

A 10-15mm vertical incision was made through the skin in the ventral region of the abdomen, immediately to the right of the midline. A similar incision was made through the muscle layer, taking care to leave the peritoneum intact. The abdominal cavity was then exposed by cutting through the peritoneum using scissors. The size of the ovaries and corpus luteum (length x width) and the width of the uterine fundus were measured using calipers. Notes were made on the presence and distribution of the corpora lutea (CL) and general ovarian appearance.

Approximately 0.3ml of blood was taken from both utero-ovarian veins using a 27 gauge needle. Following sampling, a cotton swab was pressed over the site to minimize haematoma formation.

The incisions in the peritoneum and muscle layer were closed with a continuous stitch (4/0 chromic suture; Ethicon Ltd.) and the tissue carefully aligned. After the application of Trinamide power (May and Baker) the incision in the skin was closed with a continuous subcuticular purse stitch and then with two or three loose surface stitches.

Laparotomy was repeatable within 1 week on an individual animal. In some cases, utero-ovarian vein blood was difficult to obtain in subsequent laparotomies.

2) Hysterotomy

The procedure was as described for laparotomy except that a larger abdominal incision was necessary in

later pregnancy. The gravid uterus and the ovaries were delivered through the ventral midline incision. Ovarian and uterine measurements and notes were taken. Utero-ovarian vein blood was taken from both sides.

The conceptus sac was removed, intact if possible, through a horizontal incision in the uterine wall between the two main branches of the myometrial vascular tree. The uterine incision was closed and contraction of the uterus in animals more than 50 days pregnant was aided by 0.1-0.3ml of Ergometrine (Burroughs, Wellcome & Co. Ltd.) injected into the myometrium and by a 0.1-0.25ml intramuscular injection of Syntocinon (Sandoz Products Ltd.).

Following removal of the feto-placental unit and the stitching of the uterus, approximately 0.3ml of blood was taken from each utero-ovarian vein using the same procedure as described in section 2.6-1 . The samples were taken within 15 minutes of removal of the feto-placental units. It was not possible to always obtain blood from both veins after removal.

2.7 Sampling regime for laparotomies and hysterotomies

Data obtained from laparotomies and hysterotomies are given in Chapters 4-7 and therefore a summary of the sampling regime applicable to all these chapters is given in this section (Table 2-1).

It was not considered practical to remove the conceptual sac prior to day 30 without hysterectomy. Laparotomies

Table 2-1. A summation of the sampling regime for laparotomies (L) and hysterotomies (H) and the samples obtained. T = timed; E = estimated; R & L UOV = right and left utero-ovarian veins; A.F. = amniotic fluid; U.V. = umbilical vein; F.S. = fetal serum; 1,2,3 indicates the no. of fetuses samples obtained from; Plac. Cult = placental cultures; Plac. Cont = placental content.

Day	T or E	L or H	R & L UOV		No. Fetuses	A.F.	U.V.	F.S.	Plac. Cult.	Plac. Cont.
			Pre	Post						
1	T	L	+							
5	T	L	+							
5	T	L	+							
5	T	L	+							
10	T	L	+							
10	T	L	+							
20	T	L	+							
20	T	L	+							
30	T	H	+	+	2				+	
30	E	H	+	+	2				+	
40	T	L	+							
40	T	L	+							
40	T	H	+	+	2				+	+
40	E	H			2				+	+
50	T	H	+	+	2				+	+
50	T	H	+	+	2				+	+

Table 2-1 continued.

Day	T or E	L or H	R & L UOV Pre	R & L UOV Post	No. Fetuses	A.F.	U.V.	F.S.	Plac. Cult.	Plac. Cont.
60	T	H	+	+	2				+	+
60	T	H	+	+	2				+	+
60	E	H	+	+	2					+
60	E	L	+							
70	T	H	+	+	2	1			+	+
70	E	H	+	+	2	1			+	+
75	E	H	+		2					
80	T	H	+	+	2	1			+	+
80	T	H			2	2			+	+
85	T	H	+		2	2				+
90	T	H	+	+	3	3	3	1	+	+
90	T	H	+		3	3	1		+	+
100	T	H	+	+	2(+1)	2		2	+	+
100	E	H			2	2	2	1	+	+
105	T	H	+	+	3	3	3	3	+	+
110	T	H	+	+	2	2	2	2	+	+

Table 2-1 continued

Day	T or E	L or H	R & L UOV Pre	R & L UOV Post	No. Fetuses	A.F.	U.V.	F.S.	Plac. Cult.	Plac. Cont.
115	E	H			1	1	1	1	+	+
120	T	H	+	+	3	3	3	3	+	+
130	T	H	+	+	2	1	3	3	+	+
130	E	H	+		3	1	2	2	+	
140	T	H	+	+	2	-	2	2	+	+
140	E	H			3	-	3	3	+	+
Total	T=28 E=10	L=11 H=27	33	18	60	28	25	23	24	23

were performed on 2 animals 3-4 days following birth. During pregnancy, a total of 11 laparotomies between days 1 and 60 were done on 6 animals. Serial laparotomies were done at days 1 and 10 and days 5 and 10 in 1 animal, and at days 20, 40 and 60 in 2 animals. A total of 27 hysterotomies were done, 18 of which were timed pregnancies. At least 1 timed pregnancy was taken at every 10 day interval between days 30 and 140. Of these 27 pregnancies, 19 consisted of twins, 7 of triplets and 1 of a singleton.

2.8 Collection and processing of samples

The conceptus was measured (prior to day 90) and then opened by carefully cutting through the chorionic sac. Amniotic fluid was collected and the amniotic sacs opened leaving vascular connections intact. Notes on the fetal and placental attachments were taken. Fetal samples were then taken.

Vascular connections were cut and the placental tissue was dissected free of membranes. The 2 placental discs were weighed and measured and then divided into pieces approximately 3mm in size. A random selection of pieces from each disc were preserved in Bouins solution for histological examination by other investigators, or were frozen immediately using dry ice and stored at -20^{o}C for hormonal content studies (Chapter 5), or weighed pieces were taken for culture studies (Chapter 5).

Fetal body and organ weights and measurements were then taken (Chapter 7.2). Embryos prior to day 90 were fixed in 10% buffered formalin. Sections of some of these embryos were prepared by the Department of Obstetrics and

Gynaecology, University of Edinburgh. Fetal organs were preserved in Bouins solution for histological studies by other investigators. A section of the fetal gonads and adrenals was also taken for culture. A sample from the amniotic membrane, fetal gonads and plasma was given to Drs. Speed and Chandley, MRC Clinical and Population Cytogenetics Unit, Edinburgh for studies on blood and tissue chimaerism that are still in progress.

3.1 Introduction

The small size of the marmoset precludes the withdrawal of large amounts of blood. In the present study, it was necessary to simultaneously measure several hormones in small smaple volumes and in several types of samples. Antisera to the hormones of interest were already available but these assays were not validated for marmoset samples. This chapter describes the application and validation of several radioimmunoassays for a variety of sample types for the marmoset.

The general radioimmunoassay procedure and validation will be given for the steroid (Sect. 3-2), LH/CG (Sect. 3-3) and prolactin (Sect. 3-5) assays. Generally, the adaptation and validation of these assays for placental tissue (Sect. 3-3, K) and cultures (Sect. 3-4) is given in separate sections.

3.2 Steroid radioimmunoassays

The steroids measured in this thesis were progesterone, oestradiol-17β, oestrone, androstenedione and testosterone. The samples included peripheral, utero-ovarian, umbilical vein and fetal plasma, amniotic fluid, placental tissue and culture media.

(a) Reagents, solvents and steroids

Unless stated otherwise, all reagents used were analar grade from BDH.

Assay buffer: 0.05M phosphate gelatin buffer. 45gm of sodium chloride, 43gm of disodium hydrogen orthophosphate, 34gm of sodium dihydrogen orthophosphate and 5gm of gelatin were dissolved in 5 litres of distilled water. Sodium thiomersalate ($0.001\%^{w}/_{v}$) was added as a preservative.

Solvents: Analar grade petroleum ether (B.P. 40-60°C), methanol, ethyl acetate, Aristar grade ethanol, analytical grade diethyl ether (BDH), hexane (BDH), iso-octane (BDH), and benzene (BDH).

Stripping agents: Activated charcoal Norit A was obtained from Sigma, and dextran T 70 was supplied by Pharmacia Fine Chemicals.

Scintillation fluid: This was prepared by adding 10gm of 2,5-diphenyl-oxazole (PPO; Koch-Light) and 750mg of p-tris-(2-(2phenyl-oxazolyl))-benzene (POPOP; Koch-Light) to 2.5 litres of toluene (analytical grade; Koch-Light) and 1.25 litres of Triton X-100 (analytical grade; Koch-Light).

Steroids: Nonradioactive progesterone, oestradiol, oestrone, androstanedione and testosterone were obtained from Sigma. Accurately weighed amounts were dissolved in ethanol and stored at 4°C.

Chromatography compounds: Celite (Johns-Manville Celite 545, U.S.A.), Alumina (Savory & Moore, London), Sephadex LH-ZO (Pharmacia, Fine Chemicals).

Radioactive steroids: The radioactive hormones used were: 1,2,6,7-^{3}H-progesterone; specific

activity (s.a.) 180μCi/mg (Radiochemical Centre, Amersham); 6,7-^{3}H-oestradiol-17 ; s.a. = 45-50μCi/mM (New England Nuclear: N.E.N.); 2,4,6,7-^{3}H-oestrone; s.a. = 98μCi/mM (N.E.N.); 1,2-^{3}H-testosterone; s.a. = 40-45μCi/mM (N.E.N.); and 1,2-^{3}H-androstenedione; s.a. = 60μCi/mM (N.E.N.). All radioactive steroids were diluted to a concentration of approximately 10μCi/ml in ethanol and stored at 4°C.

Antiserum: The details for the steroid antiserum are given in Table 3-1. The conjugate, the species the antiserum was raised in, the major cross reactions, the reference for a complete description of the antiserum and the person who kindly supplied the antiserum is given.

(b) Extraction of steroids from plasma and amniotic fluid.

A range of sample volumes (0.01 to 0.10ml) was used for extraction depending on the expected concentration of steroid. All samples were made up to 0.10ml with phosphate gelatin buffer. Progesterone, oestrogens and androgens were extracted separately. The 2 oestrogens were extracted separately for the peripheral plasma samples.

Progesterone was extracted with 1.0ml petroleum ether, oestradiol and oestrone with 1ml diethyl ether, and androstenedione and testosterone with 1.0ml of hexane ether (4 volumes to 1 volume). Petroleum ether and hexane were distilled not more than 24 hours before use.

Table 3-1. Details of the antiserum for the progesterone (P4), oestradiol-17β (OE2), oestrone (OE1), androstenedione (A) and testosterone (T) assays. OE3 = oestriol; bovine serum albumin = BSA.

	Conjugate	Species	Cross-reactions	Reference	Source
P4	11α-hydroxyprogesterone-BSA	Rabbit	11α-hydroxy-progesterone 36%, 17-hydroxy-progesterone 1.4%, pregnenolone 0.05%, A 0.03%	Dighe & Hunter,	Dr. K. Dighe, Edinburgh
OE2	oestradiol 6-(O-carboxymethyl)oxime-BSA	Rabbit	OE1 3.0%, OE3 0.4%, T 0.003%, P4 0.002%, A 0.002%	Baird et al, 1976	Dr. D. Baird, Edinburgh
OE1	oestrone-6-(O-carboxymethyl)oxime-BSA	Rabbit	OE2 0.08%, OE3 0.011%, P4, A, T 0.001%	Rowe, Cook & Dean, 1973	Dr. P. Rowe, Liverpool
A	11α-hydroxy-4-androstene-3,17-dione hemisuccinate-BSA	Rabbit	11β-hydroxy-androstenedione 36%, androsten-one 43%, T 0.3%	Baird et al, 1974	Dr. W. Schopman, Rotterdam
T	testosterone-3-(O-carboxymethyl)oxime-BSA	Goat	5α-dihydro-testosterone 25% OE2 0.20%, A 0.08%	Corker & Davidson, 1978	Dr. S. Tillson, Alza Corporation U.S.A.

Diethyl ether was washed with 50% ($^{w}/_{v}$) ferrous sulphate (BDH) in 5% ($^{w}/_{v}$) sulphuric acid (BDH) and distilled water (1l. of diethyl ether to 50ml acidified ferrous sulphate solution and 200ml of water) and distilled not more than 24 hours before use.

The hormones were extracted by mixing the tubes (75 x 15mm, Gallenkamp) vigorously in a multi-vortex mechanical shaker (Baird and Tatlock) for three minutes. When the aqueous and solvent phases had separated, the aqueous phase was frozen quickly by placing the tubes into methanol containing dry ice.

The solvent phase was then decanted into 75 x 12mm glass tubes (Kimble) for the progesterone, oestradiol and oestrone assays. These tubes were placed in a heated block (Driblock DB3, Tecam) and the solvent evaporated to dryness under a regulated flow of nitrogen. The extract for the testosterone and androstenedione assay levels was placed directly on alumina columns (see section 3.2f).

(c) Estimation of procedural losses

The recovery of hormone from plasma and amniotic fluid was estimated for each hormone by the addition of tracer amounts of radioactive progesterone (5pg, ~2000cpm), oestradiol (7pg, ~1000cpm), oestrone (5pg, ~1000cpm), androstenedione (5pg, ~1000cpm). Samples were equilibrated with the tracer hormones for a minimum of 30 minutes at room temperature. At the same time as aliquots were taken for radioimmunoassay, an aliquot was

taken for recovery estimation. 3 20µl aliquots of the tracer hormone (total counts) were also taken.

A mean (±s.d.) recovery of labelled hormones of 68±2% (n=100), 85±1% (n=100) and 83±2% (n=100) was found for progesterone, oestradiol and oestrone respectively. This mean recovery figure was used to correct for procedural losses for progesterone and oestradiol in peripheral plasma samples taken between the 1st and 12th week of pregnancy. There was no significant difference ($P<0.05$, paired t-test) using the mean recovery figure or the actual recovery figure for progesterone (n=100) or oestradiol (n=100).

Procedural losses were estimated individually in all other samples for progesterone and oestradiol and in all samples for oestrone, androstenedione and testosterone. The mean (±s.d.) recovery figure for oestradiol and oestrone in 50 plasma samples in which a celite chromatography step was included was 75±5% and 70±7% respectively. The mean (±s.d.) recovery figures for 50 plasma sampels of androstenedione and testosterone assay were 77±4% and 72±3% respectively.

Differing amounts of plasma for extraction or differing amounts of buffer for redissolving the dried extract did not affect the recovery figure. (Table 3-2)

(d) Radioimmunoassay of progesterone

The dried residue from the extraction procedure was re-dissolved in 0.3ml of buffer. The contents of the tubes were mixed thoroughly on a vortex mixer and

Table 3-2a. The percentage (±s.e.m. n=3) of the radioactive tracer recovered from differing volumes of marmoset plasma.

	Progesterone	Oestradiol
100µl	80 ± 2	92 ± 2
50µl	77 ± 1	94 ± 2
20µl	81 ± 2	90 ± 1
10µl	82 ± 1	90 ± 1
5µl	79 ± 1	93 ± 2

Table 3-2b. The percentage (±s.e.m. n=3) of radioactivity recovered from the extraction of 20µl plasma which had been redissolved in differing amounts of buffer. Each figure is a mean of triplicate determinations.

	Progesterone	Oestradiol
100µl	70 ± 2	83 ± 1
300µl	72 ± 3	85 ± 1
500µl	69 ± 3	83 ± 2
1ml	70 ± 2	86 ± 2
2ml	68 ± 3	85 ± 2

were incubated for at least 1 hour. After mixing, duplicate 100µl aliquots were transferred to glass assay tubes (75 x 10mm; Kimble: Corning). 50µl aliquots were transferred simultaneously to counting vials for recovery determination (see 3-2c).

For each assay a standard curve was constructed. Standards were diluted in buffer and dispensed in triplicate so that tubes contained 15-1000pg of progesterone in a volume of 100µl. 100µl of antiserum in buffer (initial dilution 1:10,000) and 100µl of 1,2,6,7-^{3}H-progesterone in buffer (10,000cpm, 25pg) were added to all tubes containing standard and unknowns, to give a final incubation volume of 300µl. In addition, triplicate sets of total counts (TC), non-specific binding (NSB) and total bound (TB) tubes were set up as follows:

TC	:	^{3}H-progesterone	(100µl)
NSB	:	^{3}H-progesterone	(100µl)
		buffer	(200µl)
TB	:	^{3}H-progesterone	(100µl)
		antiserum	(100µl)
		buffer	(100µl)

Tubes were either incubated overnight at 4^{o}C or at room temperature for at least 1 hour, and then placed on ice. The free and bound fractions were separated with a suspension of dextran-coated charcoal in buffer (25mg. dextran and 250mg. charcoal per 100ml. of buffer). 1.0ml of this suspension was added to all tubes, except the TC tubes which received 1.0ml buffer. The tubes were

mixed and left on ice for 15 min. All tubes were centrifuged at 2500rpm for 10min. at 4°C, and the supernatants immediately decanted into counting vials. Following the addition of 10ml. of scintillation fluid, the vials were allowed to equilibrate in a cooled (4°C) scintillation counter (β-particle radiation detector; Packard, Model 3375) for 30-60min. before counting.

(e) Radioimmunoassay of oestradiol-17β and oestrone

No celite chromatography step was used (Baird et al, 1974) for peripheral plasma samples. A comparison of results from samples taken throughout pregnancy (paired t test) for oestradiol ($P>0.05$; n=12) and oestrone $P>0.05$; n=10) with and without celite chromatography showed that chromatography was not necessary for these samples. (Table 3-3)

The high values of oestrone relative to oestradiol in the utero-ovarian vein, umbilical vein and fetal plasma, amniotic fluid and placental extracts necessitated the use of a celite chromatography step. The method used was described by Baird et al (1974). Essentially, the dried residue was redissolved in 0.5ml iso-octane, which had been distilled no more than 24 hours prior to use. The samples were then transferred to celite columns prepared as described by Abraham, Tulchinsky and Korenman (1970). A further 5.5ml iso-octane was applied to the column. Oestrone was then eluted with 4ml of 15% (15:85v/v) ethyl acetate in iso-octane and then oestradiol was eluted with 35% (35:65v/v) ethyl acetate in

Table 3-3. A comparison of values (ng/ml) obtained for oestrone and oestradiol in marmoset plasma with and without celite chromatography.

Oestradiol		Oestrone	
No chromatography	Celite chromatography	No chromatography	Celite chromatography
0.90	1.12	5.46	6.04
1.61	1.47	8.26	9.75
20.60	23.60	13.40	12.20
50.00	41.70	23.35	30.62
94.05	100.90	42.10	43.10

iso-octane. The eluates containing oestradiol and oestrone were evaporated.

The dried residue from the peripheral plasma extracts and celite columns was redissolved in 0.1 to 0.5ml of buffer. Duplicate 0.1ml samples were taken for radioimmunoassay and 0.05ml for recovery estimation. Samples in which no radioactive traces had been added for recovery estimation were assayed singly as they had been extracted in duplicate.

The remainder of the radioimmunoassay procedure was carried out as described for progesterone apart from the following differences: Oestradiol and oestrone standards were diluted to concentrations of 8-500pg/100µl buffer. Approximately 5000cpm/100µl of radioactive oestradiol-17β (35pg) and oestrone (25) were added to the appropriate assay. The oestradiol antiserum was diluted 1:8000/100µl and the oestrone antiserum 1:6000/100µl. 30-50% of the labelled steroid hormones were bound using this antiserum dilution.

(f) Radioimmunoassay of androstenedione and testosterone

The hexane-ether extracts of androstenedione and testosterone were transferred to prepared alumina columns (Furuyama, Mayes and Nugent, 1970). The column separation was essentially as described by McNatty et al (1976) with minor modifications to the column elution. After placing the extract on a previously washed alumina column, the column was rinsed with 4.8ml 0.2% ethanol in

hexane (Et-Hex). Androstenedione was then eluted with 2.4ml 0.2% Et-Hex. A further 5.4ml 0.2% Et-Hex was run through the column before eluting testosterone with 3.2ml 1% Et-Hex. A clear separation of the 2 hormones was obtained with this elution. (Figure 3-1)

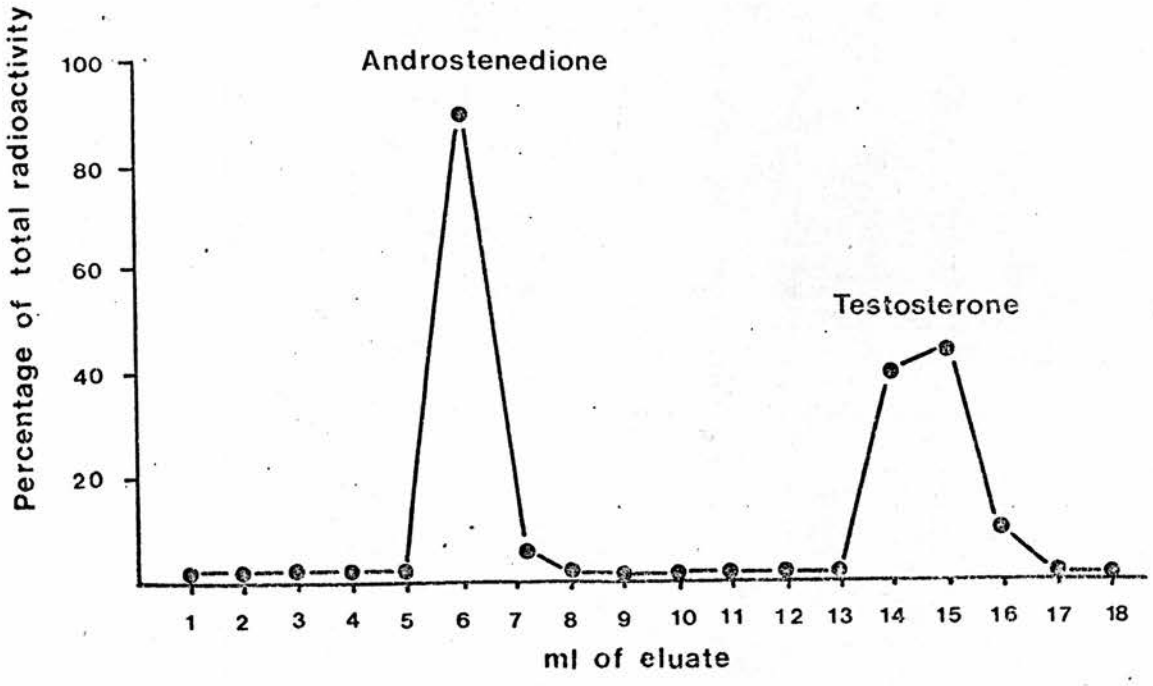
The elutes were evaporated and the dried residue was redissolved in 0.25 to 0.50ml of buffer. Duplicate 0.1ml aliquots were taken for radioimmunoassay and 0.02 to 0.10ml aliquots for recovery estimations.

The radioimmunoassay procedure for adrostenedione and testosterone was similar to that for progesterone except in the following ways: Testosterone standards were diluted to concentrations of 12.5-400pg/100µl buffer and adrostenedione standards to 8-1000pg/100µl buffer. About 5000cpm of radioactive testosterone (30pg) and androstenedione (30pg) was added to the appropriate assay. The testosterone antiserum was diluted 1:8000/100µl and the androstenedione antiserum 1:15000/100µl. 40-50% of the labelled steroid hormones were bound using these dilutions.

(g) Calculations

A program for a desk computer (9821A Calculator Hewlett Packard) was written to construct a straight line from the typically sigmoid standard curve by Mr. R. Sharpe. A standard curve was calculated using a Y axis of the logit transformation of B/Bo, and an X axis of the pg values of the standards on a log scale at a dose interval of 2. Values for logit B/Bo versus dose of standard were plotted by the computer. The calculation of

Fig. 3-1: The elution pattern for radioactive androstenedione and testosterone on alumina columns.



a standard curve omitted the points>90% of Bo and<10% of Bo, and a straight line of best fit for the data points was drawn. Steroid concentrations were calculated by the computer as pg/tube. The results were then corrected to give ng/ml plasma.

The program gave values for the 90%, 50% and 10% binding and the slope of the line. This allowed inter-assay comparison of the standard curves, which was important when new standards were prepared. The coefficient of variation for 10 assays for the 90% and 10% binding and the slope of the line was less than 5% for all hormones. The assays were chosen over the whole range of time during the study.

(h) Assay sensitivity

The sensitivity of the assays (the precision of the measurement of zero (Bo)) was for practical reasons defined as the mass of hormone required to suppress the binding of the labelled hormone to 90% of the binding achieved with no hormone added (B/Bo). The assay sensitivity (pg/tube and pg/ml) is given in Table 3-4. The sensitivity of the assay per sample (pg/ml) varied, depending on the dilution used. The minimum detection limit was not always obtained due to insufficient sample.

Buffer blanks were extracted in duplicate with every assay and were >90% B/Bo for all hormones. Plasma from ovariectomized marmosets and from male marmosets were also extracted. The values for progesterone, oestradiol-17β and oestrone were > 90% B/Bo. Male

Table 3-4. The assay sensitivity (mean ± s.d., n = 10) for the steroids measured in marmoset plasma.

	Assay sensitivity	
	pg/tube	pg/ml
Progesterone	15 ± 2	1470
Oestradiol-17β	9 ± 2	211
Oestrone	14 ± 4	1010
Androstenedione	16 ± 3	1000
Testosterone	15 ± 3	300

marmoset testosterone values were a mean of 27±5ng/ml (s.d. n=10).

(i) Inter-assay and intra-assay precisions

The inter-assay and intra-assay precisions were expressed as the coefficient of variation for replicate determinations of 2 pools of marmoset plasma and are given for the 5 steroid hormones in Table 3-5. These precisions apply to assays containing plasma and amniotic fluid samples.

(j) Assay validations

The steroid assays were validated as follows:

(1) There was no significant departure from parallelism between standards and serial dilutions of marmoset plasma (peripheral, utero-ovarian vein, umbilical vein, and fetal plasma), amniotic fluid, culture media or placental extracts for progesterone, oestradiol, oestrone androstenedione or testosterone ($P>0.05$ to $P>0.2$) using 2-factor analysis of variance with replication. (ANOVAR)

(2) The recovery of non-radioactive ligand added to pooled samples of plasma, amniotic fluid, culture media and placental tissue gave regression lines and correlation coefficients as shown in Table 3-6. No systematic errors in the methods were indicated.

(3) The concentrations of oestradiol-17β and oestrone in marmoset plasma and placental tissue were confirmed by gas-chromatography-mass-spectrometry (GCMS) (Kelly, 1971; Kelly and Taylor, 1976). GCMS was done by

Table 3-5. The inter-assay and intra-assay coefficients of variations (n = 10) for 2 pools (1 and 2) of marmoset plasma for the steroid hormones measured in this study.

Hormone	ng/ml		Coefficient of variation			
			Inter-assay		Intra-assay	
	1	2	1	2	1	2
Progesterone	30	160	9	10	5	3
Oestradiol-17β	0.5	70	13	10	7	5
Oestrone	8	20	11	12	6	7
Androstenedione	12	8	15	13	9	9
Testosterone	2	1	14	12	10	8

Table 3-6. The regression lines and the correlation coefficient for the recovery of non-radioactive ligand added to samples $P < 0.01$ in all cases.
The amount of hormone added was 1.2 - 125ng/ml for progesterone, 0.3 - 125ng/ml for oestradiol, 1.6 - 24.7ng/ml for oestrone, 0.48 - 12ng/ml for androstenedione and 0.3 - 9.6ng/ml for testosterone. $n \geq 6$ for each regression calculation.

	Plasma	Amniotic fluid	Culture Media	Placental Tissue
Progesterone	y=0.985x+0.451 r=0.999	y=0.954x+0.351 r=0.980	y=0.979x+0.500 r=0.998	y=0.910x+0.300 r=0.995
Oestradiol-17β	y=0.978x+0.526 r=0.999	y=1.078+0.489 r=0.997	y=0.995+0.460 r=0.997	y=1.102x+0.390 r=0.950
Oestrone	y=1.008x+0.006 r=0.998	y=1.201+0.008 r=0.950	-	y=0.950x+0.105 r=0.999
Androstenedione	y=1.024x+0.007 r=0.997	y=0.985+0.010 r=0.999	y=1.031x+0.059 r=0.980	y=1.046x+0.080 r=0.997
Testosterone	y=1.155x+0.274 r=0.950	y=1.100+0.200 r=0.980	y=0.96x+0.100 r=0.970	y=1.079+0.301 r=0.998

Dr. R. Kelly. Radioimmunoassay values for plasma oestradiol-17β were 116 and 110ng/ml and GCMS values were 106 and 106ng/ml. The values for plasma oestrone were 10.3ng/ml (radioimmunoassay) and 9.7ng/ml (GCMS). The values for placental oestradiol-17β were 47ng/gm (radioimmunoassay) and 50ng/gm (GCMS), and for placental oestrone, 62ng/gm (radioimmunoassay) and 57ng/gm (GCMS).

Progesterone was not compared because of the instability of the derivative required for GCMS. Testosterone and androstenedione were not compared because the low plasma levels would have required a large amount of plasma to be used for GCMS.

(4) Other investigators did not find a chromatography purification step necessary for the progesterone antiserum in other species (K.Dighe, I.Clarke, K.Henderson, pers comm) It was desirable to confirm this for marmoset samples. Table 3-7 shows the values obtained with and without the addition of a Sephadex LH-20 step. There was no significant difference in the values obtained by the 2 procedures (paired t-test, $P>0.05$, $n=13$) indicating that a purification step was not necessary.

The method used for LH-20 chromatography was as described by Carr, Mikhail and Hicklinger (1971) and by the Bulletin on Sephadex LH-20 (1970). A solvent mixture of heptane: benzene: methanol (85:10:5) (S-3) was used and the elution pattern of Carr et al (1971) was confirmed by the application and recovery of cholesterol, progesterone, pregnenelone, androstenedione and testosterone.

Table 3-7. A comparison of values (ng/ml or ng/gm) obtained for progesterone in marmoset samples with and without Sephadex LH-20 chromatography.

		No chromatography	Sephadex LH-20
Peripheral	1	55	62
	2	180	165
Utero-ovarian	1	1250	1340
	2	2380	2204
Umbilical vein	1	928	901
	2	315	392
Fetal	1	502	475
	2	274	268
Amniotic fluid	1	151	160
	2	300	320
Placental tissue	1	80	75
	2	2650	2789
	3	1890	1900

The progesterone (11-14ml) fraction was thereafter collected. An extract of buffer gave >90% B/Bo indicating no solvent blank from the Sephadex columns.

(k) Steroid concentrations in placental tissue

Several investigators have described methods for the extraction of steroids from placental tissue. (Diczfalusy and Troen, 1961; Leung and Solomon, 1972; Diczfalusy and Lindkvist, 1956; Telegdy, Weeks, Wiquist and Diczfalusy, 1970; and others). These methods were established before the advent of the more specific radioimmunoassay procedures and numerous separation and purification steps were necessary. In addition tissue quantities well in excess of 10 grams were used and probably presented problems that have been avoided for the marmoset by the use of less than 0.08 grams of tissue.

The following procedures, which were validated for marmoset placental tissue only, demonstrate a fairly simple method for placental steroid extraction and purification prior to measurement by radioimmunoassay.

Placental tissue was stored at -20°C in small pieces following hysterotomy (see Chapter 2-8). All procedures prior to steroid extraction were done in an ice bath. A minimum of 3 homogenates per placental disc were done on tissue obtained after day 60 of pregnancy. 2 to 4 homogenates were obtained from placental tissue at 40-50 days of pregnancy. Weighed amounts of tissue (0.03 to 0.08 grams) were hand homogenized with

0.8ml of phosphate gelatin buffer. Microscopic examination of the homogenate showed that the cells were broken up. The homogenate was transferred to accurately calibrated (for 2.0ml) centrifuge tubes. The homogenisers were rinsed 3 times with a total of 0.6ml of buffer and the rinses were added to the appropriate centrifuge tube. The tubes were then centrifuged at 1000rpm at $4^{o}C$ for 5 minutes to get rid of the foam caused by the homogenisation procedure.

The volume in all the homogenates was made up to 2.0ml. The homogenate was mixed well and 0.2ml (10%) was removed for the progesterone assay, 0.5ml (25%) for the oestradiol and oestrone assays and 1.0ml (50%) for the androstenedione and testosterone assays.

The recovery of each hormone was estimated after this stage for each placental homogenate by the addition of tracer amounts of the appropriate radioactive steroid (see section 3.2a,c).

Procedural losses were not estimated prior to this stage as it was desirable to use the same homogenate stock for all steroids. Any steroid loss prior to this stage was shown to be minimal. No measurable amount of progesterone was being left in the homogenisers. Also, the recovery figure for progesterone was not significantly different when tracer was added at the beginning or at the normal point in the proceedings.

Following equilibration with the tracer, the progesterone homogenates were extracted with 2.0ml

petroleum ether, the oestrogen homogenates with 5ml diethyl ether and the androgen homogenates with 10.0ml of hexane ether (4:1, v/v). No emulsions formed as long as the volume of organic solvent was at least 10 times the volume of the buffer homogenate. The extraction of equivalent quantities of buffer with equivalent amounts of solvent showed that these larger volumes of organic solvents did not introduce a high 'blank' value. In all cases, the buffer extracts were >90% B/Bo.

Three homogenates for each hormone were extracted a 2nd and 3rd time to check the completeness of the 1st extraction. In all cases, less than 3% of the total radioactivity added was recovered with additional extractions. In addition, radioimmunoassay of progesterone values in the 2nd and 3rd extract showed values less than 2% of those obtained in the 1st extraction. Therefore it seemed that the extraction of radioactivity paralleled the endogenous hormone in the tissue, and that only 1 extraction of the placental homonogenate was necessary.

A similar percentage of radioactive tracer was recovered irrespective of the amount of tissue used. The percentage of progesterone radioactive tracer recovered was 75-80% from tissue concentrations of 0.01 to 0.08 grams (n=8). The percentage of oestradiol, oestrone, androstenedione and testosterone radioactive tracer recovered from 5 homogenates prior to the chromatography step was 80 to 90% for all 4 hormones. This indicated that a substantial

amount of the hormone was being extracted from the tissue despite differences in the amount of tissue used.

Most authors utilised a centrifugation step of the placental homogenate prior to hormone extraction. The value of this was assessed. 2 placental homogenates for each hormone were equilibrated with radioactive tracer and then centrifuged at 3000rpm for 30 minutes at 30°C. The supernatant and precipitant were extracted for each hormone. The amount of radioactivity recovered in each fraction is shown in Table 3-8.

Radioimmunoassay of the amount of hormone in the supernatant and precipitant extracts showed a similar ratio for the endogenous hormone in the supernatant and precipitant as the percentage ratio for the recovery of radioactive hormone (Table 3-8).

This data indicated that the 2 most fat soluble hormones, progesterone and oestrone (Kelly, R. pers. comm.) were being trapped in the precipitant. However, the hormones could be extracted from both the precipitant and supernatant with the appropriate organic solvent. The extraction of radioactivity from the precipitant and supernatant reflected the extraction of the endogenous placental hormone.

Either a finer homogenate or extraction of the whole homogenate (with no centrifugation) were 2 alternatives to overcoming the problems with progesterone and oestrone. Since the latter was the easier solution, this was tested first and assay validations were done without a

Table 3-8. The percentage recovery of radioactivity from an extract of the placental supernatant and precipitant following centrifugation and of buffer.

	Supernatant	Precipitant	Buffer
Progesterone	56	26	92
Oestradiol-17β	91	< 3	85
Oestrone	66	20	88
Androstenedione	90	3	83
Testosterone	86	3	80

centrifugation step.

After obtaining an extract of the placental homogenates, the procedures for radioimmunoassay were essentially as described in sections 3.2d,e & f. The procedures are summarised at the end of this section (3.2k1).

The coefficient of variation (CoV) for 10 placental homogenates prepared from 1 placental disc was 5%, a similar figure to the intra-assay CoV for plasma samples (Table 3-5). This showed that the small piece of placental tissue used for homongenisation was fairly representative of the overall placental hormonal concentration.

The inter-assay CoV was determined by replicate determinations of a placental homogenate and was similar to values in Table 3-5 for all hormones.

The mean value for each placental homogenate was calculated (using 2-4 replicates). 3 mean placental homogenate values were used to calculate the mean value per placental disc (except for the 40-50 day values).

(1) Summary of method

1) Weigh and homogenise tissue with 0.8ml buffer. Centrifuge 1000rpm for 5 minutes. Make volume to 2.0ml. Mix.

2) Take 0.1ml for progesterone assay

   a) Add progesterone tracer and equilibrate

   b) Extract with 1ml petroleum ether

   c) Dry extract and redissolve in 0.3-1.0ml buffer.

d) Take 0.05-0.2ml for recovery determinations.

e) Take duplicate 0.01 and 0.10ml for radioimmunoassay

f) Assay as in section 3.2d.

3) Take 0.5ml for oestrogen assays

a) Add oestradiol and oestrone tracers. Equilibrate

b) Extract with 5ml diethyl ether and dry extract

e) Redissolve residue in iso-octane and apply to celite columns. Collect oestradiol and oestrone fractions. Dry

f) Redissolve in 0.25-0.05ml buffer

g) Take 0.02-0.1ml for recovery determinations

h) Take duplicate 0.02-0.10ml for radioimmunoassay.

i) Assay as in section 3.2e.

4) Take 1.0ml for androgen assays

a) Add androstenedione and testosterone tracers. Equilibrate

b) Extract with 10ml hexane:ether (4:1,v/v)

c) Reduce extract to about 1.0ml

d) Apply extract to alumina columns and collect androstenedione and testosterone fractions. Dry.

e) Redissolve in 0.25-0.30ml buffer

f) Take 0.02-0.05ml for recovery determinations

g) Take duplicate 0.10ml for radioimmunoassays

h) Assay as in section 3.2f.

(2) Summary of validations for placental tissue

1) There was no significant departure from parallelism between standards and serial dilutions of placental extracts. (See section 3.2k).

2) The recovery of known amounts of added hormone showed no systematic error in the method. (Table 3-7)

3) There were good recoveries of radioactive tracers for all hormones from placental homogenates with a range of tissue amounts. The recovery of the radioactive tracers reflected the recovery of endogenous hormone.

4) Progesterone values were similar in placental extracts with and without Sephadex LH-20 chromatography (Table 3-8). Oestradiol and oestrone levels were confirmed by GCMS (see section 3.1j).

5) Intra-assay precision (utilising individual homogenates) was within the normal range. This also indicated that the small piece of tissue used was probably fairly representative of the overall placental hormone concentration.

6) Larger volumes of solvents used did not interfere with the assay.

3.3 Radioimmunoassay of luteinising hormone/chorionic gonadotrophin (LH/CG)

The majority of the samples measured for LH/CG were from pregnancy. In the present study, marmoset LH/CG was measured by adaptation of the heterologous double-antibody radioimmunoassay for marmoset LH. (Hodges, 1977, 1978). The assay used NIAMDD-rat LHI-1 for iodination, NIAMDD-rat LHRP-1 for standards and anti-ovine LH610V for antiserum. (Dr. J. Uillenbrock, Amsterdam) There were problems over parallelism between marmoset pregnancy samples and the NIAMDD-rat LHRP-1 standards. As there is not yet available a standard preparation for marmoset CG, serial dilutions of an extract from a 60 day marmoset placental tissue were used as a standard. (See section 3.3h)

The assay cross-reacted with NIAMDD-rat LHRP-1 by 3%, with bovine FSH (6FSH CH-1-76) and NIAMDD-rat FSH I-1 by 0.17% and $< 0.3\%$ respectively, and with all human preparations by less than 0.05%. (Hodges, 1977, 1978.)

(a) Reagents

Buffers

Phosphate buffered saline (PBS; 0.01M, pH 7.8):

This buffer was made from stock solutions of 0.4M disodium hydrogen orthophosphate (A) and 0.4M sodium dihydrogen orthophosphate (B). 36gm. of sodium chloride, 91.6ml. of A and 8.4ml. of B were made up to 4l with distilled water. 0.04gm. of sodium thiomersalate was added as a preservative.

Phosphate buffered saline (PBS; 0.05M, pH 7.8): This buffer was prepared in an identical way to the one above except that the solution was made up to 800ml.

Phosphate buffered saline plus bovine serum albumin (PBS + BSA): BSA was added to 0.01M PBS to give a concentration of 1gm. per 100ml. (1% BSA).

"Special buffer": 3.72 gm. ethylenediamin-etetra-acetic acid (EDTA; Sigma) was dissolved in 1l. of 0.01M PBS. The pH of this solution was adjusted to 7.5 using 3N. sodium hydroxide. Normal rabbit serum (6.7ml. NRS; Wellcome Reagents Ltd.) and 2gm. BSA were added.

Barbitone buffer (0.12M; pH 8.5): Diethyl barbituric acid (110gm.) was dissolved in 4.5l of distilled water, and 19gm. of sodium hydroxide in 1l. of de-ionised water was added and stirred for 2 hours. The solution was then made up to 5l. with distilled water and stirred for 24 hours.

Barbitone buffer plus BSA: 5gm. BSA were dissolved in 100mg. Barbitone buffer (i.e. 5% BSA).

All buffers were stored at $4^{o}C$.

(b) Iodination of rat LH

Rat LH preparation NIAMDD-rat LHI-1 was labelled with $Na^{125}I$ (Radiochemical Centre, Amersham) by a modification of the chloramine-T method of Greenwood, Hunter and Glover (1963). All iodinations were done by Dr. J. K. Hodges and the method used has been fully described. (Hodges, 1977, 1978). For each assay, the label was diluted to 10000cpm in 100µl PBS + 1% BSA.

(c) Antiserum

Anti-ovine LH610V, raised in rabbits was stored at -20°C. For each assay, the antiserum was diluted 1:70,000 with special buffer.

(d) Standards

Standard LH solutions were prepared from 50µl aliquots (10mg. hormone) stored at -20°C by diluting with PBS + 1% BSA buffer to concentrations of 0.6-160ng/100µl.

Standard placental solutions for pregnancy samples were prepared from dilutions of the supernatant of a 60 day marmoset placental homogenate. This stage was chosen for the combination of high hormonal values and sufficient quantities of tissue.

LH/CG was extracted from placental tissue by homogenisation of tissue (0.02-0.15 grams) in 3ml. of 0.01 molar PBS. The homogenate was shaken for 3 hours in a mechanical shaker (Gallenkamp) and then centrifuged at 3000rpm for 30 minutes at 4°C. The supernatant was decanted and aliquots were removed for serial dilution with PBS + 1% BSA for the standard solutions. The top dose was assigned the value of 100 units and each dose was calculated from this value.

The same method was used for extracting LH/CG from all placental tissue samples.

(e) Assay procedure

All samples were assayed in duplicate using plastic 63 x 11mm. tubes. (Sarstedt).

The protocol of each assay included:

1) Total count tubes (TC):

^{125}I-LH (100µl).

2) Non-specific binding tubes (NSB) :

special buffer (100µl);

PBS + 1% BSA (200µl);

^{125}I-LH (100µl).

3) Total bound tubes (TB) :

PBS + 1% BSA (200µl);

antiserum (100µl);

^{125}I-LH (100µl).

4) Standards :

Standard LH or standard placental extract (100µl)

antiserum (100µl);

^{125}I-LH (100µl).

5) Unknowns and quality controls :

Samples (plasma, placental extracts or culture media) + buffer (PBS + 1% BSA) (total volume = 200µl);

antiserum (100µl);

^{125}I-LH (100µl).

Standards or unknowns and antiserum was added on the first day. The tubes were mixed and incubated at 4°C for 3 days. ^{125}I-labelled LH was added to all tubes which were mixed and incubated at 4°C for a further 2 days.

Separation of antibody bound and free hormone was achieved by adding 200µl of donkey anti-rabbit gamma globulin (1:30 $^{v}/_{v}$, in 0.01M PBS) (Burroughs Wellcome, RD 17)

and incubation was continued at 4°C for 12 hours. The unbound radio-activity was diluted with 1ml 0.01M PBS and the tubes centrifuged at 4°C for 30 minutes at 2500 rpm. The supernatants were discarded, the tubes dried with tissue paper, and the antibody-bound ^{125}I-labelled LH in the precipitate was measured in an automatic gamma spectrometer (Wallac Decem - GT2).

(f) Precisions and sensitivity

The inter-assay CoV was 4% for repeated assays (n = 5) of a pool of marmoset pregnancy plasma. The detection limit of the assay for placental standards was 1 unit/tube; 20 units/ml for pregnancy plasma samples and 185 units/gram for placental tissue. The detection limit for LH standards was 1ng/tube and 20ng/ml for plasma. These limits were not always obtained due to insufficient sample.

(g) Calculations

A typically sigmoid standard curve was obtained using Y-axis of B/Bo and an X-axis of values of the standards on a log scale at a dose interval of two. The curve was drawn to pass through the mean value of each dose of standard. LH standards were used for samples taken around ovulation and placental standards for all pregnancy samples.

(h) Assay validation

There was no significant departure from parallelism ($p > 0.2$, ANOVAR) between NIAMDD LHRP-1 standards,

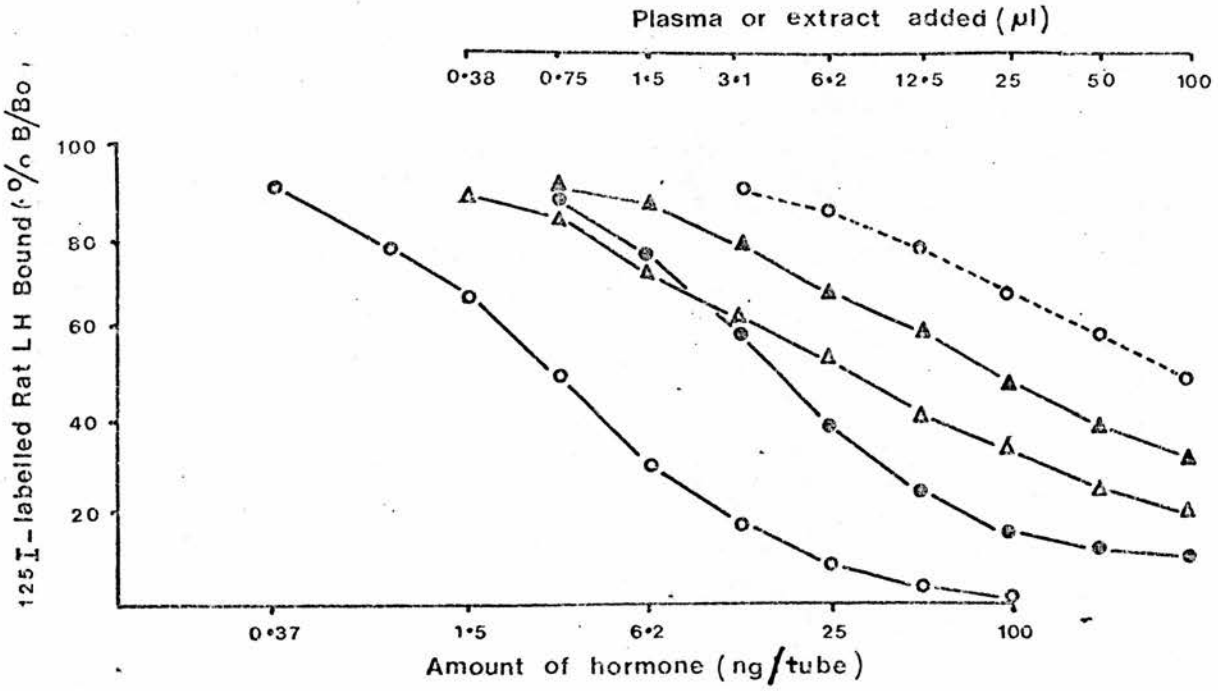
non-pregnancy plasma and marmoset pituitary extracts between 25 and 87% B/Bo. There was also no significant departure from parallelism between serial dilutions of marmoset peripheral and utero-ovarian vein pregnancy plasma and placental extracts or between 3 placental extracts (days 40, 60, 75) ($p > 0.2$, ANOVAR). Placental tissue and pregnancy plasma were not parallel to the NIAMDD LHRP-1 standards, non-pregnancy plasma or pituitary extract.

A comparison of some of the inhibition curves obtained for these samples is given in Figure 3-2. Estimation of pregnancy samples using LH standards would have resulted in a large underestimation of gonadotrophin values. In view of these findings and the lack of an alternative standard for marmoset pregnancy samples, it was decided to use serial dilutions of a marmoset placental extract for all pregnancy samples.

The placental extract could be assayed in a biological system (e.g. radio-receptor assay, Parlow - ovarian ascorbic acid depletion assay, OAAD) to assign a potency to these standards which would allow quantitative comparison to other species.

The parallelism between pregnancy plasma and placental extracts and the non-parallelism with non-pregnancy plasma and pituitary extracts indicated a distinct 'pregnancy' gonadotrophin with a placental source. Sephadex chromatography should confirm a clear separation of the immunological fractions between the non-pregnant

Fig. 3-2: Inhibition curves for NIAMDD-rat LHRP-1 (○—○), pooled peripheral (▲) and utero-ovarian (○···○) plasma from pregnant marmosets, a marmoset placental extract (△) and pituitary extract (●).



and pregnant state. In addition, a biologically and immunologically active gonadotrophin (CG) from the placenta of the marmoset was demonstrated. (Hobson, 1972) which cross-reacted biologically (Hobson and Wide, 1972) and to a lesser extent immunologically (Hodgen et al, 1976) with hCG.

Although absolute confirmation of the assay must await purification of a marmoset CG preparation, the utilisation of the present assay system gave comparative CG levels during pregnancy for the marmoset.

3.4 Radioimmunoassay of culture media

(a) Progesterone, oestradiol, androstenedione and testosterone

Culture media were not extracted prior to radioimmunoassay for progesterone (McNatty, Henderson and Sawers, 1975). The direct assay of culture media was not assessed for the other hormones as relatively few samples were measured.

Duplicate 0.01-0.1ml aliquots of culture media or of diluted culture media were taken directly for assay (progesterone, section 3.2d) or for extraction, chromatography and assay (sections 3.2e & f).

Validations were as follows:

1) 2 standard curves, with an extra 0.1ml of buffer or 0.1ml culture media, were superimposable for all hormones.

2) There was no significant difference in progesterone values (paired t-test, $p>0.05$, $n=6$) for extracted

and non-extracted samples.

3) There was no significant departure from parallelism between standards and placental cultures (ANOVAR, $p>0.05$).

4) Recovery of added hormone indicated no systematic error in the method. (See Table 3-7.)

(b) LH/CG

The measurement of LH/CG secreted into culture media by placental tissue followed the protocol described in section 3.3. There was no significant departure from parallelism between placental standards and serial dilutions of placental culture media (ANOVAR, $P>0.05$). The addition of culture media (0.1ml) to the standard curve had no effect.

3.5 Radioimmunoassay for prolactin

Marmoset prolactin was measured using a heterologous double-antibody radioimmunoassay described in detail previously (McNeilly and Friesen, 1978; McNeilly, Abbott, Lunn, Chambers and Hearn, 1981). The assay uses a guinea-pig anti-human prolactin antiserum (33-9) with ^{125}I-labelled ovine prolactin as tracer and ovine progactin (NIH-P.S6, 26iu/mg) as standard. The assay cross-reacted with prolactin preparations from the sheep, cow and pig by 80% and more, with dog (Schering) by 79%, with rabbit (McNeilly 76-1-MC) by 17% and with rat NIAMDD P-I-1 by $< 0.1\%$. Growth hormone, placental lactogen, LH and FSH preparations from many species all cross-reacted by less than 0.1%

(McNeilly and Friesen, 1978). All prolactin assays were done by Dr. A.S.McNeilly.

(a) Reagents

0.01M PBS, 0.05M PBS and PBS + 1% BSA :

identical to the recipes in the LH assay (section 2.16a(i)).

0.1% and 1%-Tris-HCl buffer (0.025M; pH 7.5) :

3.025gm/l of 2-amino-2-(hydroxymethyl)propane-1,3-diol, (tris) (Tris(hydroxymethyl) methylamine; BDH) and 1gm/l (0.1%) or 10gm/l (1.0% of BSA were dissolved in distilled water and the pH adjusted to 7.5 with hydrochloric acid (HCl).

Physiological saline (0.9% ($^{W}/_{V}$) NaCl; pH 7.2) :

9gm of sodium chloride (NaCl) were added to every litre of distilled water.

Iodination of ovine prolactin

Ovine prolactin (o PRL) NIH-P-S-6 was labelled with $Na^{125}I$ (Radiochemicals Centre, Amersham) by the lacto-peroxidase method (Thorrel and Johansson, 1971) at room temperature, as described previously (Shin, Kelly and Friesen, 1973). The ^{125} labelled prolactin was stored at -20°C until used for assay, when it was diluted to 20,000cpm in 100µl PBS + 1% BSA.

(b) Standards

Standard prolactin solutions were prepared from 20µg of the hormone stored at -20°C in 1ml of 0.1% BSA-Tris-HCl buffer. Dilutions with 0.01M PBS were prepared in the range of 0.4-200ng/ml.

(c) Antiserum

Anti-human prolactin 33-9 was stored at -20°C in 1ml aliquots at a dilution of 1:100. For each assay this was diluted to an initial concentration of 1:6000 with PBS and 1% BSA. This dilution of antiserum bound between 30 and 50% of the labelled tracer (McNeilly and Friesen, 1978).

(d) Assay procedure

All samples were assayed in duplicate using plastic 63 x 11mm tubes (Sarstedt). The protocol of each assay included:

1) Total count tubes (TC):
   ^{125}I - PRL (100µl).
2) Non-specific binding tubes (NSB):
   PBS & 1% BSA (450µl);
   ^{125}I - PRL (100µl).
3) Total bound tubes (TB):
   PBS & 1% BSA (350µl);
   antiserum (100µl);
   ^{125}I - PRL (100µl).
4) Standards:
   standard prolactin (50µl);
   PBS & 1% BSA (300µl);
   antiserum (100µl);
   ^{125}I - PRL (100µl).
5) Unknowns and quality controls:
   Plasma (50µl);
   PBS & 1% BSA (300µl);

antiserum (100µl);

^{125}I - PRL (100µl).

Standards or unknowns, PBS + 1% BSA and antiserum was added on the first day. The tubes were mixed and incubated for 24 hours. ^{125}I-labelled prolactin was added to all tubes which were mixed and incubated for 3 days at 4°C. Separation of antibody bound and free hormone was achieved by adding 100µl of normal guinea pig precipitating serum (1:600 $^{v}/_{v}$ in 0.01M PBS) and then 100µl of donkey anti-guinea pig gamma globulin (1:15 $^{v}/_{v}$ in 0.01M PBS; Burroughs Wellcome). Incubation was continued at 4°C for another 16 hours. The unbound radioactivity was then diluted with 1.0ml of physiological saline and the tubes centrifuged at 4°C for 30 minutes at 2500 rpm. The supernatants were discarded, the tubes dried with tissue paper, and the antibody-bound ^{125}I-labelled prolactin in the precipitate was measured in an automatic gamma spectrometer (Model 1270, Rackgamma; Wallac Decam).

(e) Precision

The inter- and intra-assay variation was 11% (n=10) and 7% (n=16) respectively for 3 pools of marmoset plasma containing 4, 25 and 45ng/ml.

(f) Calculations

The procedure was identical to that in the LH assay (section 3.3g).

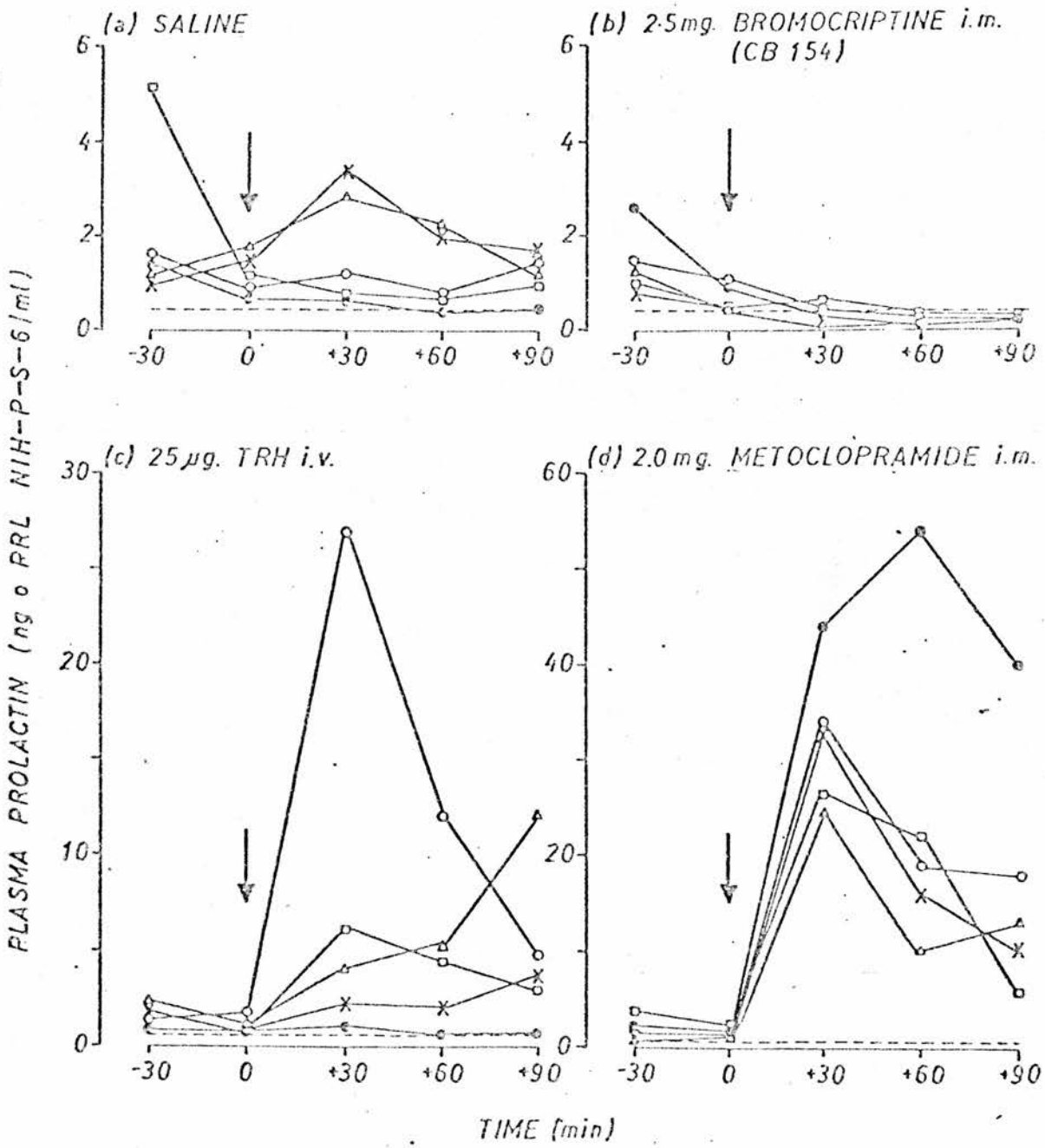
(g) Assay validation

To provide biological validation of the assay, marmosets were treated with agents known to raise (Thyrotrophin releasing hormone, (TRH; Roch) and metaclopramide (Maxalon; Beechams)) or lower (bromocriptine (CB154)) prolactin levels in other species. To evaluate cross-reaction with luteinizing hormone (LH) and follicle stimulating hormone (FSH) marmosets were also treated with luteinizing hormone releasing hormone (LHRH, Hoechst).

Five cycling female marmosets were injected with saline (0.5ml), bromocriptine (CB154) (2m5mg im) metaclopramide (2.0mg im) TRH (25ug iv) or LHRH (2ug iv). The changes in plasma prolactin levels following injection are shown in Figure 3-3. Saline and LHRH had no significant effect on plasma prolactin levels. TRH and metaclopramide caused a significant ($p<0.001$) increase in prolactin 30 minutes after injection. The increase in prolactin was significantly greater ($p<0.001$) after metaclopramide than after TRH. CB154 caused a suppression of plasma prolactin to below the sensitivity of the assay. This observation also provided evidence that marmoset plasma alone did not have a non-specific effect on the binding of ^{125}I-labelled ovine prolactin to the antiserum.

The slopes of the inhibition curves were not significantly different for ovine prolactin standards, dilutions of marmoset plasma and crude marmoset pituitary extract. Therefore all samples were assayed against, and were reported in terms of, ng ovine prolactin/ml plasma .

Fig. 3-3: Plasma levels of prolactin in female marmosets after the injection (↓) of a) saline, b) CB154 (Bromocriptine, 2.5mg im), c) TRH (25ug. i.v.) or d) metaclopramide (2.0mg im). Five animals were used and each was given all tests. The dotted line indicates the sensitivity of the assay.



CHAPTER 4 : PHYSICAL PARAMETERS AND HORMONAL PROFILES DURING THE CYCLE, PREGNANCY AND LACTATION.

4.1 Introduction

(a) Cycle

Studies on the ovarian and pituitary endocrine events during the menstrual cycle in sub-human primates, including the rhesus (Neill, Johansson and Knobil, 1967 Hotchkiss, Atkinson and Knobil, 1971; Niswender and Spies, 1973 and others) and other macaque species (bonnet: Stabenfeldt and Hendrickx, 1972; crab-eating: Stabenfeldt and Hendrickx, 1973), baboons (Stevens, Sparks and Powell, 1970; Goncharov, Aso, Cekan, Pachalia and Diczfalusy, 1976; Kling and West *et al*, 1978), chimpanzees (Reyes *et al*, 1975; Hobson, Coulston, Raiman, Winter and Reyes, 1976) and orangutan (*Pongo pygmaeus*) (Collins, Graham and Preedy, 1975) demonstrated considerable qualitative similarities among these species. These sub-human species have been considered as appropriate models for studies on the endocrinology of the human menstrual cycle.

Increasingly, New World primates are being studied as additional primate models for studies on human reproduction. Some, such as the capuchin (*Cebus apella*) (Castellanos and McCombs, 1968; Wright and Bush, 1977) and squirrel (*Saimiri sciureus*) (Wolf, O'Connor and Robinson, 1977) show regular menstrual cycles. Others, including the owl monkey (*Aotus trivirgatus*) (Elliot, Sehgal and Chalifoux, 1976; Bonney, pers. comm.), the tamarins (*Saguinus* species)

(Preslock, Hampton and Hampton, 1973) and the marmoset (Hearn and Lunn, 1975) show neither a conspicuous oestrus nor a detectable menstrual cycle; although a clearcut hormonal cycle is present.

A preliminary study in the marmoset indicated a cycle length of 17 ± 1.7 days, with a LH, progesterone and oestradiol pattern not unlike that observed for the human (Hearn and Lunn, 1975). In order to compare the hormonal profile in the nonfertile cycle with the conception cycle and with apparently long cycles (19-30 days) in several females paired with fertile males, it was necessary to use the same assay systems for the cycle data as the pregnancy data. Therefore the hormonal profile during the cycle was re-examined.

(b) Pregnancy

A preliminary study in the marmoset showed that progesterone and oestradiol increased throughout pregnancy in a manner more similar to women than to baboons or macaques (Hearn and Lunn, 1975). There is as yet no detailed analysis of the hormonal changes occurring during pregnancy for the marmoset. The first part of this study was initiated to document the overall changes occurring during pregnancy for several hormones utilising assays that were fully validated for marmoset plasma (Chapter 3). The mean trends and hormonal interrelationships were obtained by the simultaneous measurement of several hormones in samples taken throughout pregnancy. The interanimal variation was exemplified by hormonal measurements in serial samples taken from several individuals. In addition, selected stages of gestation were studied utilising a more

frequent sampling regime, but with the consequence that fewer hormones were measured. Detailed profiles of progesterone , oestradiol and LH/CG for the conception cycle, and of progesterone and oestradiol for the first 70 days of pregnancy, 80 to 100 days and the last week of pregnancy were studied in several individuals. It is intended to relate this basic data on peripheral hormone levels to ovarian and placental function (Chapter 5), fetal hormones (Chapter 6) and the pattern of prenatal development (Chapter 7).

The physical factors that were assessed were the growth of the uterus and fetal heads, the ovarian size and the number and distribution of corpora lutea.

(c) Prolactin values during lactation and the relationship to the return to fertility in primates

Lactationin women is associated with a period of amenorrhoea and infertility (see McNeilly 1979 for references) Although the exact mechanisms involved in inhibition of reproduction during the post partum period have not yet been elucidated, both elevated levels of prolactin and the suckling stimulus itself would appear to be involved. Lactation is also associated with a period of infertility in other primates; e.g. the gorilla (Gorilla gorilla) (Harcourt, Fossey, Stewart and Watts, 1980), chimpanzee (Tutin, 1980), baboon (Attman, Attman and Hausfater, 1978), rhesus monkey (Weiss, etal, 1973). Among New World primates an effect of lactation on reproduction has been observed in the spider (Ateles belzebuth) (Dempsey, 1939; Wolf, Harrison and Martin, 1975) howler (Alouatta

caraya) (Carpenter, 1934) and squirrel monkeys (Travis and Holmes, 1974; Coe and Rosenblum, 1978).

In contrast to the above species, lactation in the common marmoset monkey is not associated with any apparent post partum infertility, in spite of continued suckling by the infant for up to 100 days (Lunn and Hearn, 1978; Chambers and Hearn, 1979; Lunn and McNeilly, 1981). The aim of the present study was to determine the changes in the circulating levels of prolactin during lactation and to relate the occurrence of lactation with the return to fertility.

4.2 Procedures

(a) Uterine, ovarian and fetal head growth

One to three times weekly, the width of the uterine fundus was measured by abdominal palpation. In animals from which more than 1 measurement was taken in a week, the mean value was used. After the 15th week of gestation, the numbers and size of the fetal heads were estimated by palpation once weekly.

The width of the uterine fundus and the length (1) and width (b) of the ovary was also measured at laparotomy and hysterotomy (section 2.6a and b). The ovarian size was estimated by the formula for the volume of an oblate spheroid, $V = \frac{1}{6}\pi b^2 1$.

(b) Sampling regimes

(i) Cycle

Two animals, paired with vasectomized males, were bled daily for 30 days. A further 3 animals, paired with another female, were bled 3 times weekly for 5 weeks. Preliminary samples were taken from the latter 3 animals to establish cyclicity. It was previously shown that 1 female will continue to cycle normally when paired with another female (Hearn, 1978). The sampling duration of 5 weeks was chosen based on a previous report of the cycle length being just over 2 weeks (Hearn and Lunn, 1975). Progesterone and oestradiol were measured in all samples. LH/CG and oestrone were measured on samples when there was sufficient plasma.

(ii) Pregnancy

Samples were taken 1-3 times weekly from 5 timed pregnancies throughout pregnancy. Two animals aborted and blood sampling was discontinued. Samples were also taken from animals in whom the stage of gestation was estimated by abdominal palpation and retrospectively by the day of birth (section 2.4)

A summary of the serial weekly and 3-7 time weekly sampling regimes and the hormones measured is given in Table 4-1a and b. In some cases, the same animal that was used for the earlier part of gestation was also used for the latter part. In all cases, more than 1 hormone was measured in each sample.

Mean hormonal values for pregnancy were

Table 4-1a. The serial sampling regime during pregnancy for the 5 steroid hormones, prolactin and LH/CG. Samples were taken weekly.

Hormone	No. of animals	Duration of weekly sampling (weeks).
Progesterone	6	1-12
	9	11-20
Oestradiol	6	1-11
	9	11-20
Oestrone	3	1-7
	7	7-20
Androstenedione	3	1-9
	5	7-20
Testosterone	3	1-9
	5	7-20
LH/CG	5	1-11
Prolactin	2	1-10
	4	12-20
All of the above hormones	2	1-20

Table 4-1b. The serial sampling regime during pregnancy for progesterone, oestradiol and LH/CG. Samples were taken 3 times weekly or daily*.

Hormone	No. of animals	Duration of sampling (days)
Progesterone and Oestradiol	3	Birth-70
	5	80-100
	5	~137-Birth*
Progesterone and LH/CG	6	Birth-30
Progesterone, LH/CG and Oestradiol	2	Birth-30

calculated using all available hormonal data for each week or period of gestation. In animals from which more than 1 measurement was taken in a specified period, the mean value was used. The hormonal ratios were calculated using only samples in which both hormones were measured. Only results from pregnancies carried successfully to term were included.

(iii) Lactation

13 marmosets were bled 2-3 times weekly during the last week of pregnancy. Sampling was continued in 10 of these females, which had surviving young, for 90-100 days post partum. For the 3 remaining females, whose young had died within 5 days of birth, sampling was continued for up to 70-77 days post partum. Prolactin and progesterone were measured in all samples.

(c) Statistics

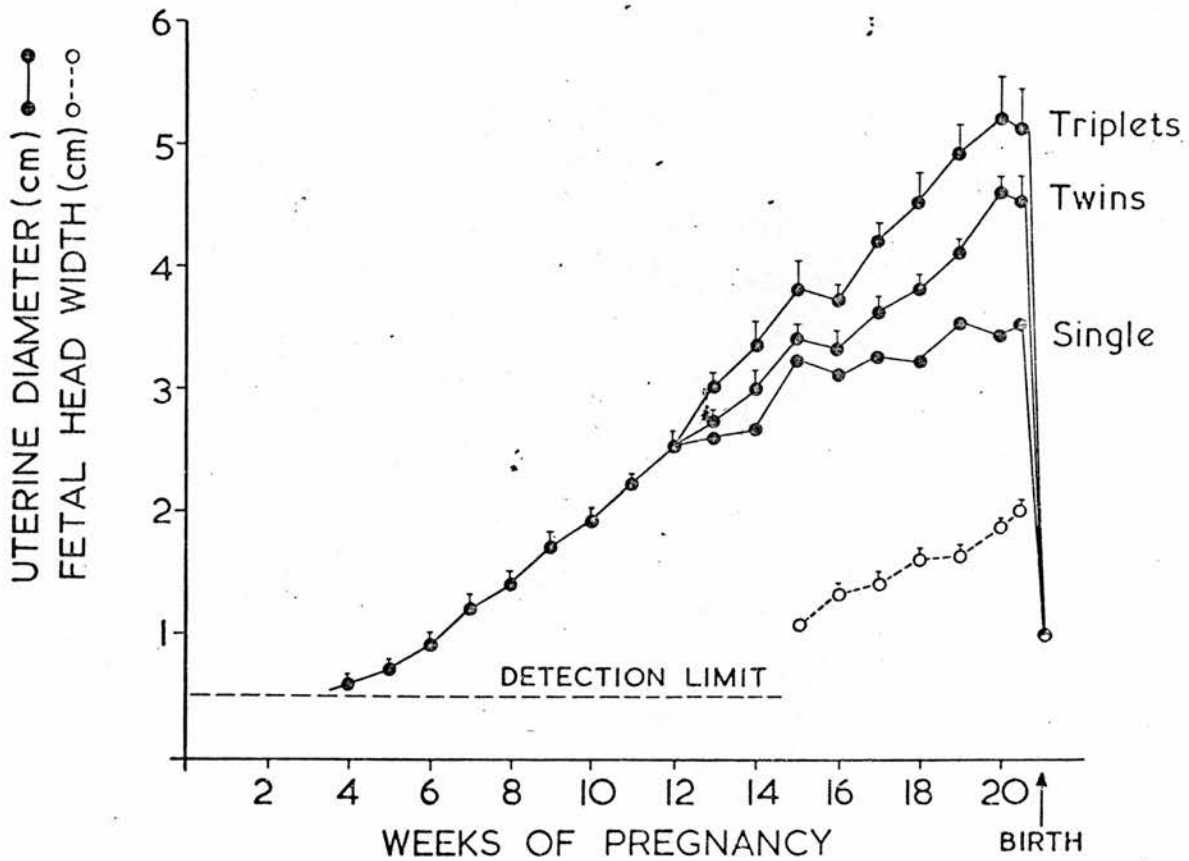
Unless otherwise stated, mean hormonal values at different stages of gestation were compared using the students t-test and hormonal values from serial samples from individual animals were compared using the paired t-test.

4.3 Results

(a) Uterine, ovarian and fetal head growth

Figure 1 shows the growth of the uterus and fetal heads for singleton, twin and triplet pregnancies. By reference to this graph for uterine growth, pregnancies of an unknown stage may be dated. There was no difference

Fig. 4-1: The diameter (±s.d.) of the uterine fundus (●) and the width of the fetal heads (o), measured by abdominal palpation in marmosets bearing single, twin or triplet young. The horizontal broken line represents the detection limit.



in the growth of the uterus between triplet, twin and singleton pregnancies until 13 weeks of gestation. A decrease in the uterine diameter was observed at 15 weeks of gestation and a decrease in the week preceeding birth when the fetal heads engage with the pelvis. Two fetal heads, as when there are twins, cannot be palpated and measured until 17-18 weeks of gestation and 3 heads, as for triplets, are not distinguishable until 19-20 weeks. Abortion was clearly indicated by a significant decrease of the uterine diameter and was confirmed by the drop in progesterone concentrations to follicular-phase levels.

Uterine and ovarian size, and the distribution and size of corpus luteum (CL) were obtained at laparotomy or hysterotomy (Table 4-2). Abdominal palpation was generally similar to the direct measurement at hysterotomy. CL were often distinguishable up to 90 days of pregnancy and were distributed on either one or both ovaries. The number of CL present generally corresponded with the number of embryos present. The ovary associated with CL was usually larger than the ovary with no CL and ovarian size was similar when both contained CL.

(b) Post-partum ovulation

Within 3 weeks of giving birth, 74% of the animals (N=31) had ovulated. The mean day of ovulation was 10.5 ± 0.7 (s.d.) days post-partum. Of the 23 animals that ovulated, 17 were suckling at least 1 young at the time of ovulation. The range (5-17 days) from birth to first ovulation was similar for both lactating and non-lactating animals.

Table 4-2. The uterine diameter (cm) measured by abdominal palpation (P) and at hysterotomy (H), the number of fetuses, the number and size (cm) of corpus luteum (C.L.) and the ovarian volume (cm) (Ov.V) for a selection of pregnancies throughout gestation. N.M. = not measured.

Gest. Stage	Uterine Size P	Uterine Size H	No. fetuses	No. C.L.	Size C.L.	Ov.V
1				1	0.3	0.079
				1	0.4	0.064
10				1	0.5	0.118
				1	0.5	0.092
30	0.6	0.7	2	1	0.4x0.6	0.118
				1	0.4x0.5	0.151
50	1.5	1.5	2	2	0.5,0.3	0.150
				0		0.025
70	1.9	1.95	2	1	0.3	0.048
				1	0.4	0.118
90	3.4	3.3	3	3	0.4,0.3,0.3	0.176
				0		0.035
110	3.7	4.3	2	1?	N.M.	0.092
						0.050
130	4.5	4.4	2	0		0.074
				0		0.046
140	5.6	5.4	2	0		0.062
				0		0.074

78% (N=18) of the ovulations resulted in pregnancy. 14 of the animals were lactating and 4 were not. Hormonal and interbirth interval data (S.Lunn, pers. comm.) showed that the remaining 5 animals conceived at the subsequent 1 or 2 cycles.

Of the 8 animals in which ovulation did not occur within 3 weeks, 5 animals were lactating and 3 were not.

After spontaneous abortion, animals resumed fertile cycles within the same time interval as those that had given birth, and 71% (n=7) of the ovulations resulted in pregnancy.

The gestation length was 144 ± 2 (s.d., N=9) days.

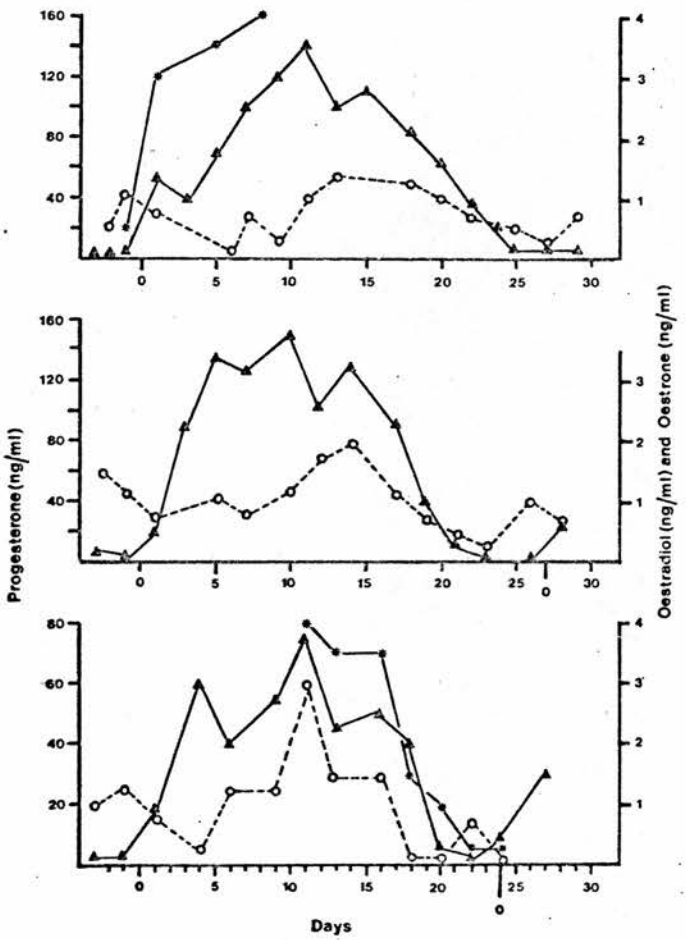
(c) The cycle

The 2 animals that were bled daily for 30 days were both acyclic as indicated by continuous progesterone values of <10ng/ml. 2 complete cycles and 1 complete luteal phase were obtained from the remaining 3 animals (Figure 4-2) which were bled 3 times weekly for 5 weeks.

The total cycle length for the 2 animals was a minimum of 24-27 days. The follicular-phase, defined as progesterone <10ng/ml, ranged from a minimum of 4-8 days. The luteal phase ranged from a minimum of 19-24 days. The mean was 21.3 ± 2.1 (s.d., N=3) days.

Maximum luteal phase progesterone values were found around day 10-12 after ovulation. Progesterone slowly declined over about the next 10 days and levels were

Fig. 4-2: Progesterone (▲), oestradiol-17β (o), and oestrone (*) during the non-fertile cycle in 3 individual marmosets. Day o = estimated day of ovulation.



lower than the normal pregnancy range (<20ng/ml) by day 19-24.

There was a rise in oestradiol just prior to the initial progesterone rise and a second rise during the luteal phase coincident or just after the progesterone peak (day 11-15). This was about 6-9 days prior to follicular phase progesterone values.

11 apparent cycles were also obtained from 11 animals paired with fertile males, who were being bled 3 times weekly for another part of this study. The duration of the luteal phase was similar to that seen in the non-fertile cycle. The mean luteal phase was 21.5 ± 2.0 (s.d., N=11) days and the range was 19-24 days. Progesterone values lower than the normal pregnancy range were reached between 17 and 22 days followed by follicular phase levels 2-3 days later. The pattern of progesterone and oestradiol was also similar to that seen in the non-fertile cycle.

Oestrone and LH/CG were also measured in some follicular and luteal phase samples. Mean oestrone follicular and luteal phase values were ≦1ng/ml (N=6) and 3.8 ± 0.5ng/ml (s.d., N=10) respectively. LH/CG was markedly elevated around ovulation in 3 samples coincident with the elevated oestradiol values. LH/CG was unmeasurable in the remainder of the samples which included samples taken during the latter part of the luteal phase from animals paired with fertile males.

(d) The post-partum period

Progesterone, oestradiol and oestrone were

similar to follicular phase levels on the day of birth following expulsion of the fetus and placenta. Since the time of birth was not generally known, the interval between birth and blood sampling was not known.

(e) Pregnancy

(i) General trends

The mean peripheral plasma levels of progesterone and LH/CG are shown in Figure 4-3, of oestradiol-17β and oestrone in Figure 4-4 and of androstenedione and testosterone in Figure 4-5. The mean concentration includes singleton, twin and triplet pregnancies as there was no significant difference for twins and triplets at any stage of gestation for progesterone, oestradiol or oestrone. Samples were obtained from only 1 singleton pregnancy. The mean concentration also includes pregnancies with male and female fetuses, all female or all male fetuses as there was no difference in hormonal levels related to fetal sex.

An example of the hormonal values for 2 individuals throughout pregnancy is shown in Figure 4-6a and b. The hormonal trends and temporal relationships will be reported with reference to the mean values. Results from serial weekly samples illustrated the interanimal variation.

(i-a) LH/CG

LH/CG rose gradually for the first 4 weeks of pregnancy and then more steeply up to the 8th week (Figure 4-3). Maximum values were found between the 8th and 10th week. Levels then gradually fell to the detection limit of the assay at 15 weeks of pregnancy and

Fig. 4-3: The peripheral plasma levels (±s.e.m.) of progesterone (▲) and LH/CG (△) during pregnancy in the marmoset monkey. The number of samples per point was 8-28 for progesterone and 5-8 for LH/CG.

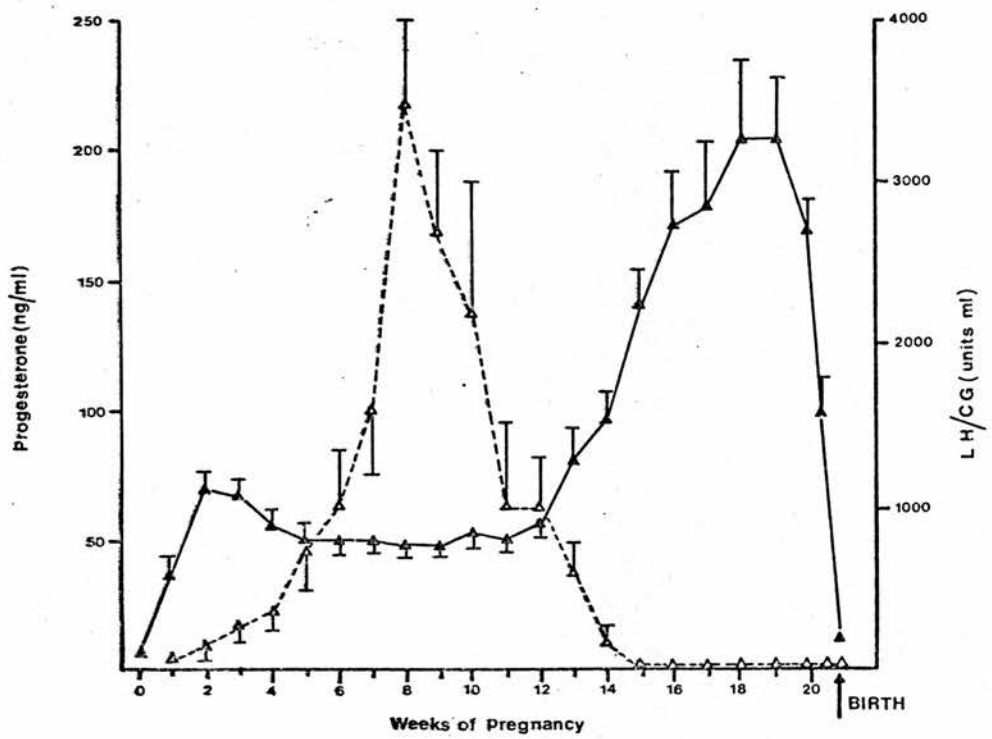


Fig. 4-4: The peripheral plasma levels (±s.e.m.) of oestradiol-17β (o) and oestrone (⁎) during pregnancy in the marmoset monkey. The number of samples per point was 10-21 for oestradiol and 6-9 for oestrone.

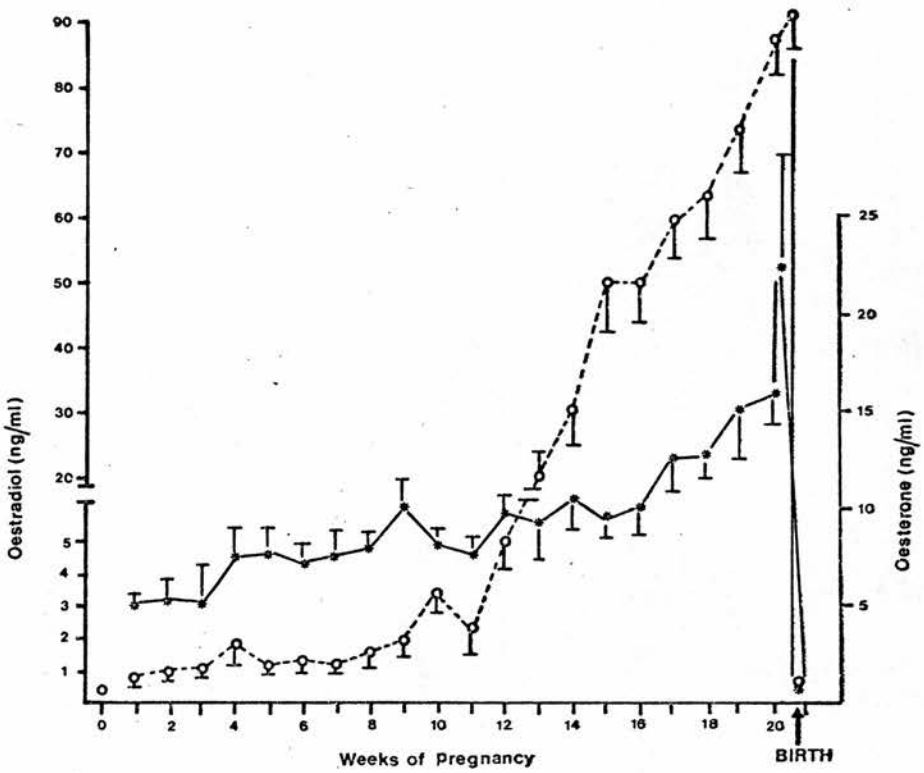


Fig. 4-5: The peripheral plasma levels (±s.e.m.) of androstenedione (■) and testosterone (●) during pregnancy in the marmoset monkey. The number of samples per point was 3-8 (except at week 1 and 20 for testosterone.

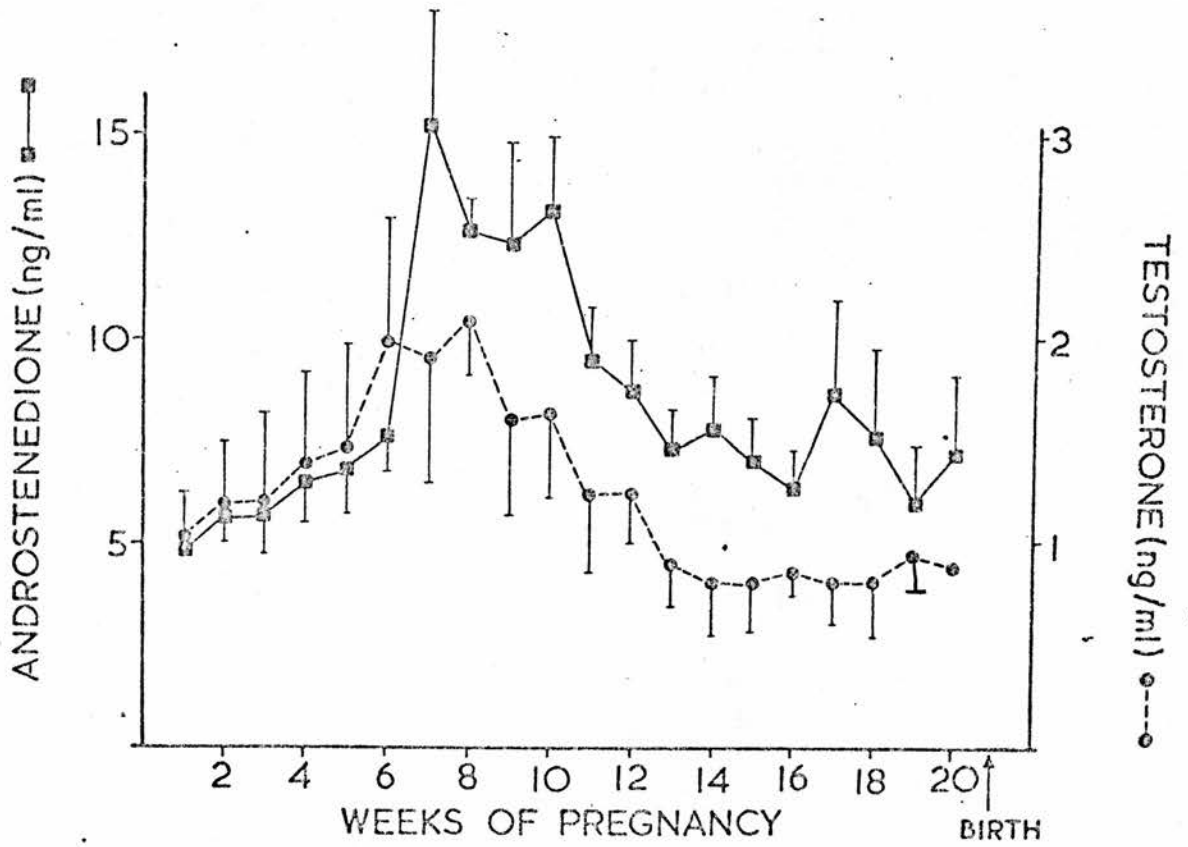


Fig. 4-6a Progesterone (▲), LH/CG (△), oestradiol (o), oestrone (✱), androstenedione (■) and testosterone (●) during pregnancy in an individual marmoset.

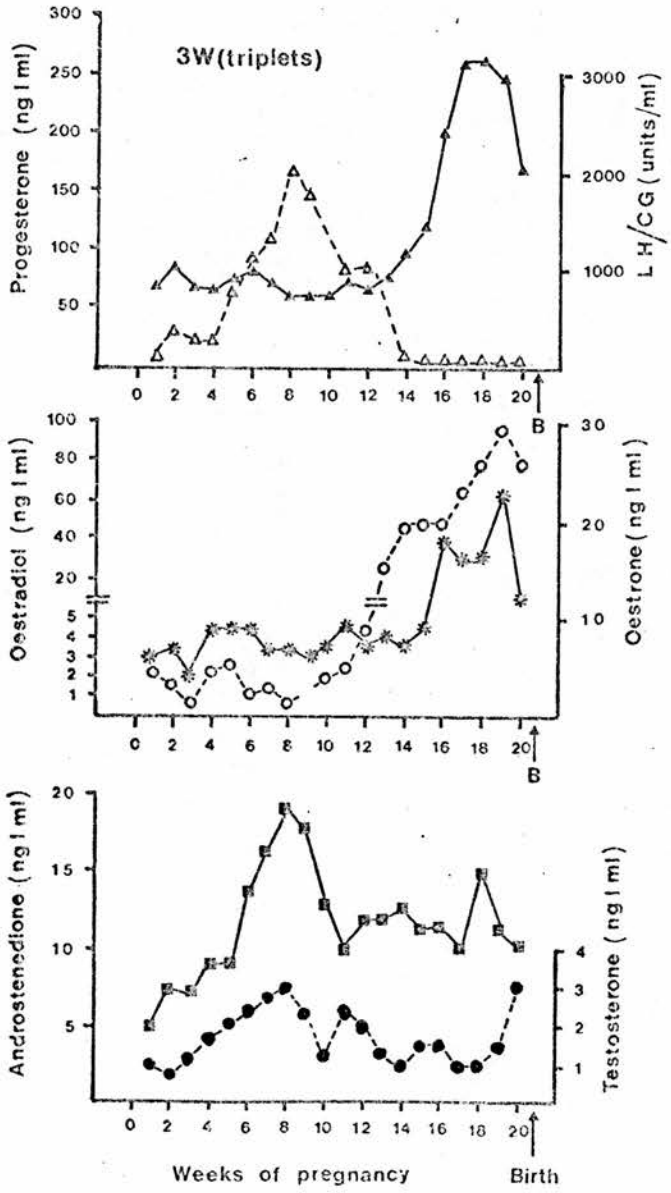
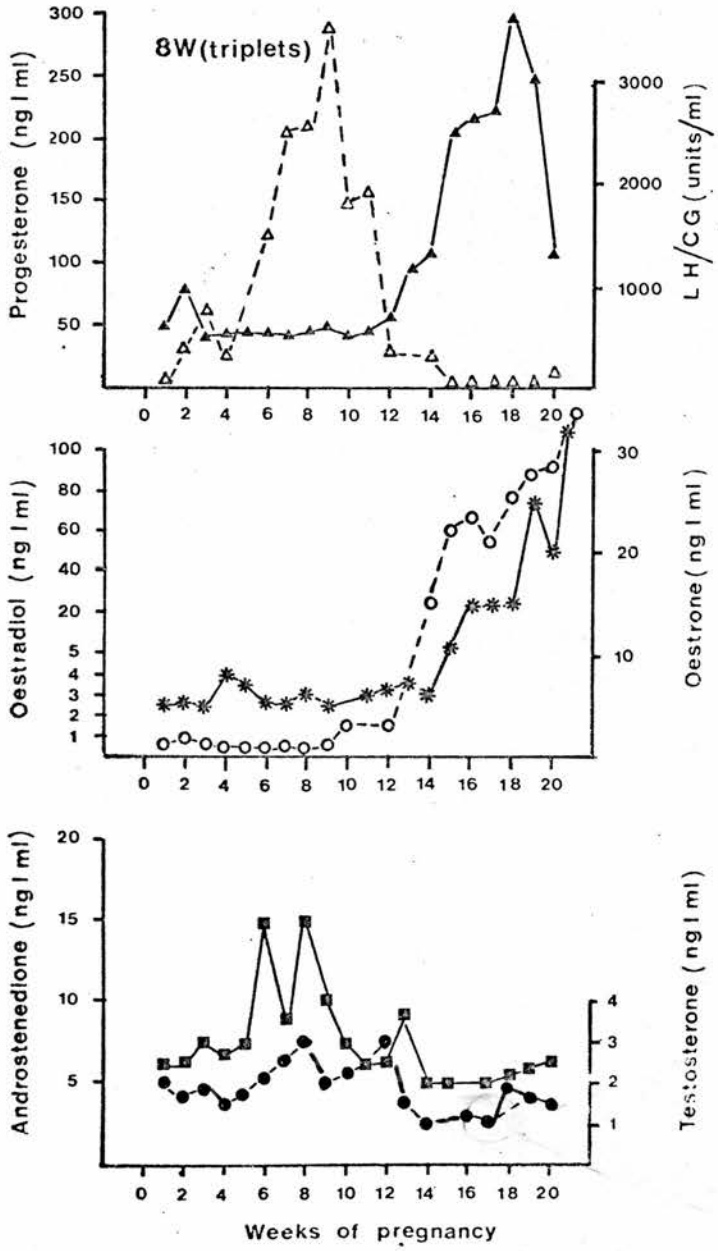


Fig. 4-6b : Progesterone (▲), LH/CG (△), oestradiol (o), oestrone (✱), androstenedione (■) and testosterone (●) during pregnancy in an individual marmoset.



remained low thereafter. There was a similar pattern in individual animals.

(i-b) Progesterone

Progesterone values rose after ovulation and maximum luteal phase levels were found by the 2nd week of pregnancy (Figure 4-3). There was a significant decrease in levels between the 3rd and 5th week ($P<0.02$) with no further significant change occurring until week 13. These stable levels were found during the period of maximum LH/CG values.

Weekly samples from 6 animals for the first 12 weeks of pregnancy confirmed a significant decline between the 3rd, 4th and 5th week ($P<0.02$) and confirmed that luteal phase levels (62 ± 5ng/ml, s.d.) were significantly higher than levels from the 4th to 12th week (48 ± 4ng/ml, s.d.) ($P<0.01$).

At 13 weeks, when LH/CG values were declining, there was a significant rise in progesterone ($P<0.01$) which continued until maximum values were reached at the 18th-19th week.

There was a decline in mean progesterone values in the $1\frac{1}{2}$ weeks preceeding birth ($P<0.01$). Levels at week $20\frac{1}{2}$ were similar to those at week 13 and less than $\frac{1}{2}$ the maximum levels.

Weekly samples from 9 animals (weeks 11-20) showed a significant increase in levels for each week for gestation between weeks 12 and 16 ($P<0.02$). Maximum values of 106-412ng/ml were found between the 15th and 18th week. These maximum levels were 2-8 times the

level found at week 11.

In these same 9 animals, progesterone declined to 50-200ng/ml in the last week of pregnancy which was 30-60% of the maximum values and was not significantly different from the values at week 13.

(ic) Oestradiol-17β

There was a 2-fold increase in mean oestradiol values during the first 9 weeks of pregnancy followed by a further 2½-fold increase between the 9th and 12th week (Figure 4-4). A rise over luteal phase values was not always observed in individuals during the first 9 weeks but by the 12th week, levels were significantly elevated over luteal phase levels ($P<0.01$, $N=6$).

At 13 weeks, coincident with the rising progesterone values, there was a steep increase in oestradiol. The increase was sustained and unlike progesterone, the maximum values were found in the last week of pregnancy.

Weekly samples from 9 animals (weeks 11-20½) showed a significant increase in oestradiol for each week between weeks 12 and 20 ($P<0.02$-0.001). Maximum oestradiol levels of 74-165ng/ml were found between 133 and 144 days. In 7 of 9 animals, the maximum value was found in the last samples taken before birth (<2 days). The maximum oestradiol values in these animals were 40-84 times the week 11 value.

More detailed description of the progesterone and oestradiol pattern during the first 70 days,

80-100 days and the last week of pregnancy is given in section 4.3e iii, iv and v.

(id) Oestrone

After ovulation, oestrone levels remained high (>5ng/ml) throughout pregnancy (Figure 4-4). There was significant increase in oestrone between weeks 3 and 4 ($P<0.02$) which was coincident with the declining progesterone and increasing LH/CG values. Levels then remained relatively constant until week 16, when levels increased until the end of pregnancy. This increase in oestrone occurred 3 weeks after the steep increase for progesterone and oestradiol.

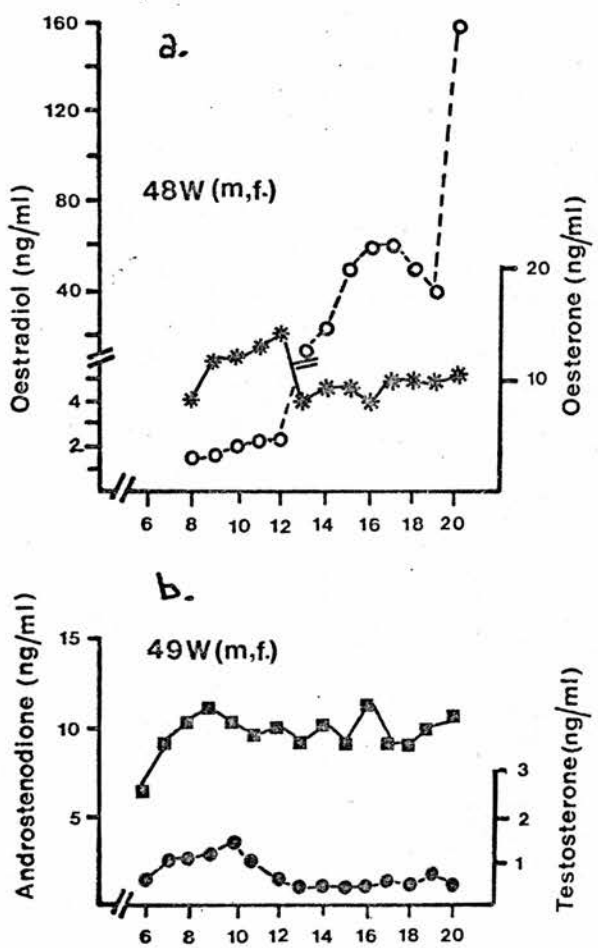
Although the mean concentration of oestrone rose in the latter part of pregnancy, a rise in oestrone values was observed in only 4 of 7 animals from which weekly samples were taken (weeks 7-20) (compare Figure 4-6 and 4-7a). Oestradiol values and the oestradiol to oestrone ratio (see section 4.3f) in all these 7 animals were within the normal range.

The magnitude of the increase in mean hormonal values from early to late pregnancy was approximately 4-fold for progesterone, 100-fold for oestradiol and 5-fold for oestrone.

(ie) Androgens

Androstenedione and testosterone both rose during the first half of gestation with the highest mean values found between 6 and 10 weeks (Figure 4-5) coincident with maximal LH/CG values. Levels

Fig. 4-7(a): Oestradiol (o) and oestrone (✱) levels in an individual marmoset from the 8th week of pregnancy.
(b): Androstenedione (■) and testosterone (●) levels in an individual from the 6th week of pregnancy.



gradually declined during the latter half of gestation to levels slightly higher (androstenedione) or similar (testosterone) to those found in early pregnancy.

Weekly samples from 3 animals confirmed a 2-3-fold increase in androstenedione and testosterone between the 1st and 9th week of gestation. Weekly samples from 5 animals (weeks 7-20) showed a decline in androstenedione values after the 9th-10th week of gestation to less than 50% of the maximum values in 3 of the animals. A decline was not evident in 2 of the animals (compare Figures 4-5 and 4-7b).

(i-f) Prolactin

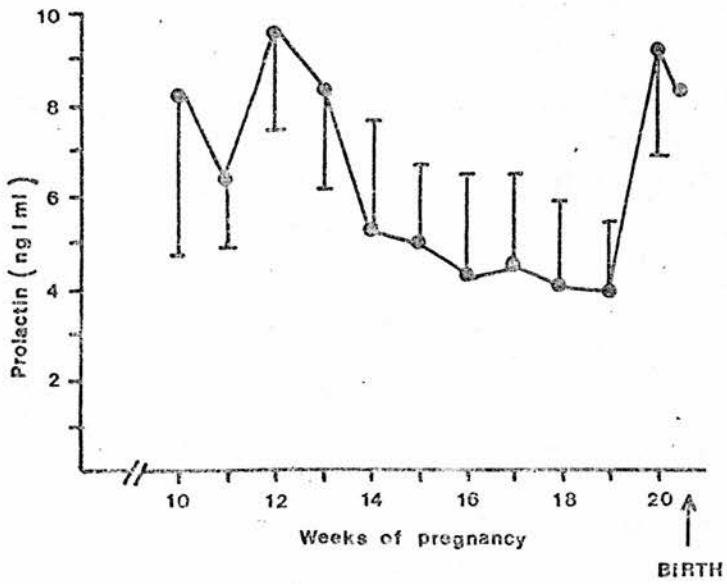
The mean prolactin levels during the second half of pregnancy are shown in Figure 4-8. There were insufficient samples from non-lactating animals during the first half of pregnancy to calculate means. Mean prolactin levels declined between weeks 12 and 19 ($P<0.05$) and increased between weeks 19 and 20 ($P<0.02$). Individual animals showed a similar prolactin profile.

Prolactin was measured in 2 non-lactating animals during the first and latter half of pregnancy. Levels during the latter half of pregnancy were similar to those in the first half. Inasmuch as there was insufficient plasma to measure prolactin at weeks 11 and 12, a rise in prolactin at this stage of gestation was not confirmed for these animals.

(ii) Hormonal ratios

The progesterone to oestradiol ratio

Fig. 4-8: Mean (±s.d.) prolactin levels during the second half of pregnancy in the marmoset.



(Figure 4-9) declined during the first 14 weeks of gestation. This was during the period of stable progesterone values and gradually increasing oestradiol values. During the 13th to 18th weeks, the ratio remained relatively constant (mean 4.3 $\pm$ 0.3 s.d.) and there was a positive correlation between these 2 hormones (regression analysis: $y = 0.232\ x + 4.705$, $r = 0.74$, $n = 57$). This corresponded to the period when both hormones were increasing. During the last 1½ weeks of pregnancy, the ratio declined nearly 4-fold. This was due to the decline in progesterone values and the continuance of high oestradiol values.

The oestradiol to oestrone ratio was relatively constant during the first 9 weeks of gestation (Figure 4-10). A gradual increase between the 9th and 12th week was followed by a steep increase between the 12th and 15th week of gestation. During the first 12 weeks of gestation, oestradiol levels were always less than 50% of oestrone levels; whereas after the 12th week, oestradiol levels always exceeded oestrone levels. This was due to the marked increase in oestradiol values which occurred during this time. There was a decline in the oestradiol to oestrone ratio during the last ½ week of gestation.

The androstenedione to testosterone ratio (Figure 4-11) gradually increased with advancing gestation. Although both hormones showed a similar pattern during pregnancy, the increase in androstenedione was relatively greater than for testosterone.

Fig. 4-9: The ratio of progesterone to oestradiol (±s.d.) during pregnancy in the marmoset.

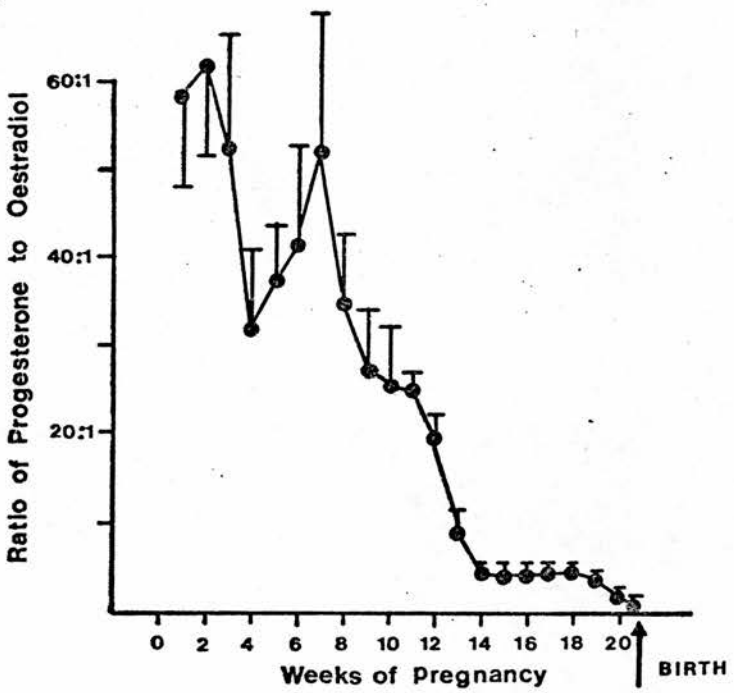


Fig. 4-10: The ratio of oestradiol to oestrone (±s.d.) during pregnancy in the marmoset.

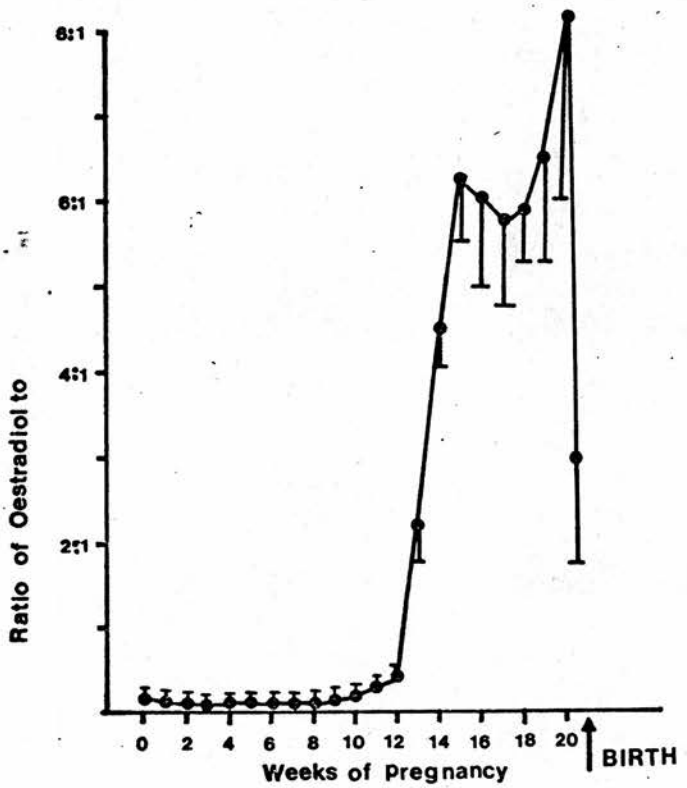
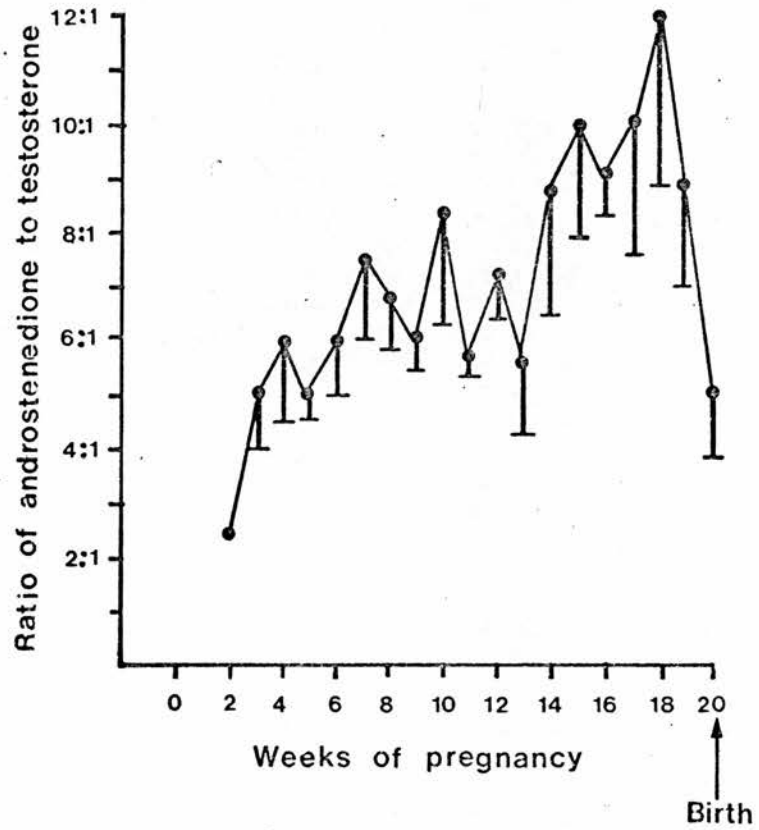


Fig. 4-11: The ratio of androstenedione to testosterone (±s.d.) during pregnancy in the marmoset.



(iii) The first 70 days

Mean progesterone and oestradiol values were calculated for 2 day intervals for the first 70 days of pregnancy (Figure 4-12). The pattern for 3 individuals is shown in Figure 4-13.

Maximum mean luteal phase progesterone values were found at day 10-11 followed by a decline to a nadir at day 29-30. In individual animals, there was either an immediate decline after day 10-12 or fairly constant levels for up to 12 more days (day 22 of the conception cycle). A decline was most obvious if luteal phase levels were high (Anim. 51W). In all cases, there was a nadir in progesterone between 24-30 days.

Although mean progesterone levels were significantly lower than luteal phase levels after the 30th day, individual values fluctuated over a range of 22-70ng/ml and sometimes exceeded luteal phase values. There was no consistent temporal relationship between animals in the peaks and troughs of progesterone.

There was a luteal phase rise in oestradiol just after the maximum progesterone levels. Levels thereafter remained high. Oestradiol values varied considerably within an individual and there were occasional peaks (4-8ng/ml) and troughs ($\lesssim$0.21ng/ml) in levels.

These oestradiol peaks were confirmed by reassay and by the addition of a celite chromatography step. Measurement of oestrone in these samples showed no comparable increase.

Fig. 4-12: Mean (±s.e.) progesterone (Δ) and oestradiol (o) levels for the first 70 days of pregnancy.

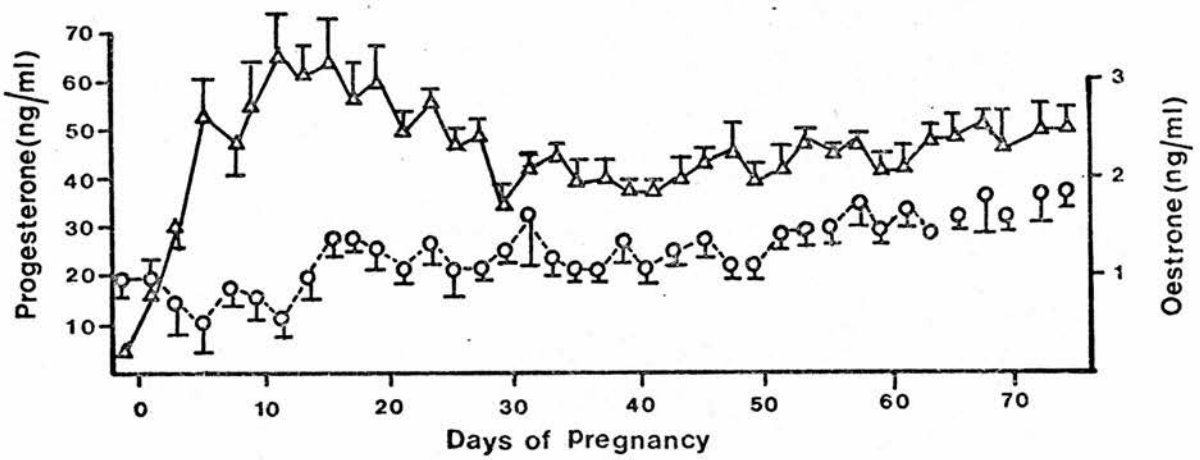
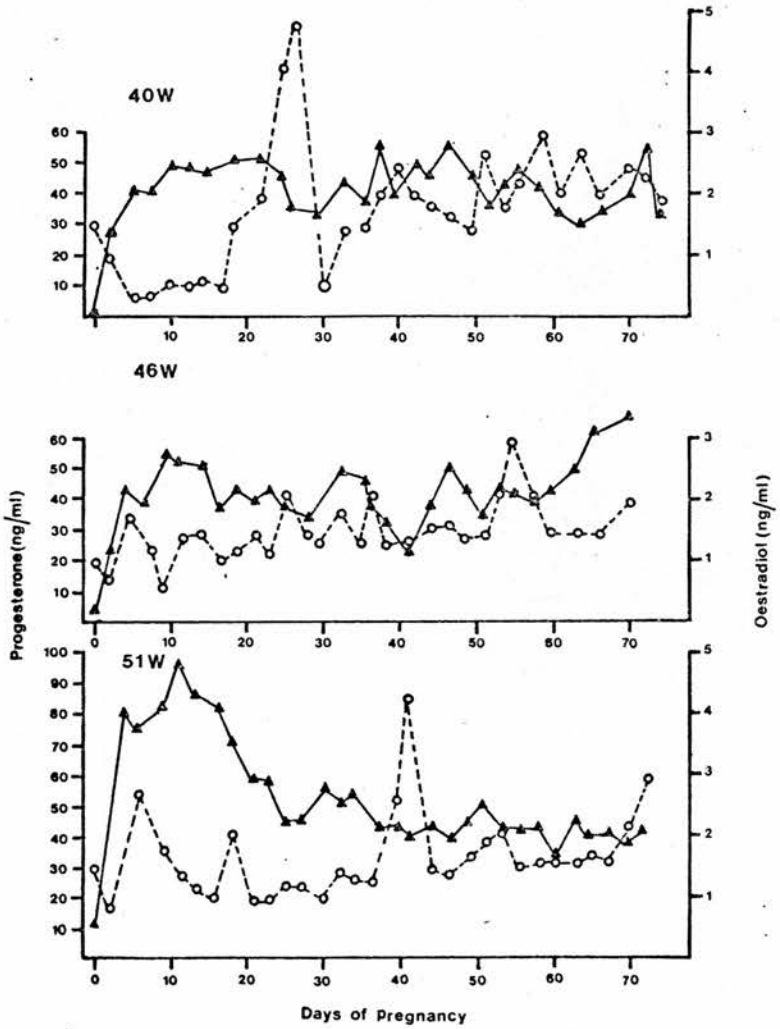


Fig. 4-13: Progesterone (▲) and oestradiol (o) values for the first 70 days of pregnancy in 3 individual marmosets.



Oestradiol and progesterone peaks and troughs were not positively or negatively associated with each other.

LH/CG was also measured in 6 animals during the first 30 days. Simultaneous with the high values of LH/CG (100ng/ml) and oestradiol (>0.5ng/ml) around ovulation, were elevated progesterone values (10-15 ng/ml) in 3 of the 6 animals. There was a second sustained rise in LH/CG 13-21 days following ovulation, which was coincident with a levelling or a decline in progesterone values in these animals, (see Figure 4-14).

(iv) 80-100 days

Weekly samples showed a steep increase in progesterone and oestradiol during this phase of pregnancy. This period was therefore examined more closely. Mean values and the progesterone:oestradiol ratio is given in Figure 4-15. The first significant rise in progesterone and oestradiol occurred simultaneously at 90-91 days. The increase for oestradiol was relatively greater than for progesterone as reflected in the declining ratio. Within this 20 day period, the overall increase was about 3-fold for progesterone and 50-fold for oestradiol. Oestradiol values >10ng/ml were never found prior to day 88 and values <10ng/ml were never found after day 92. Progesterone values prior to day 90 were variable and an increase was best observed by serial sampling.

(v) The last week

Mean and weekly serial samples indicated a decline in progesterone and possibly an increase in

Fig. 4-14: The LH/CG (Δ), oestradiol (O) and progesterone (▲) profiles for 2 animals for the first 30 days of pregnancy. LH/CG (□) measured around ovulation utilized anti-ovine LH610V for antiserum and NIAMDD-rat LHRP-1 for standards. LH/CG (Δ) measured after day 10 utilized placental extracts for standards. See Chapter 2-3.3

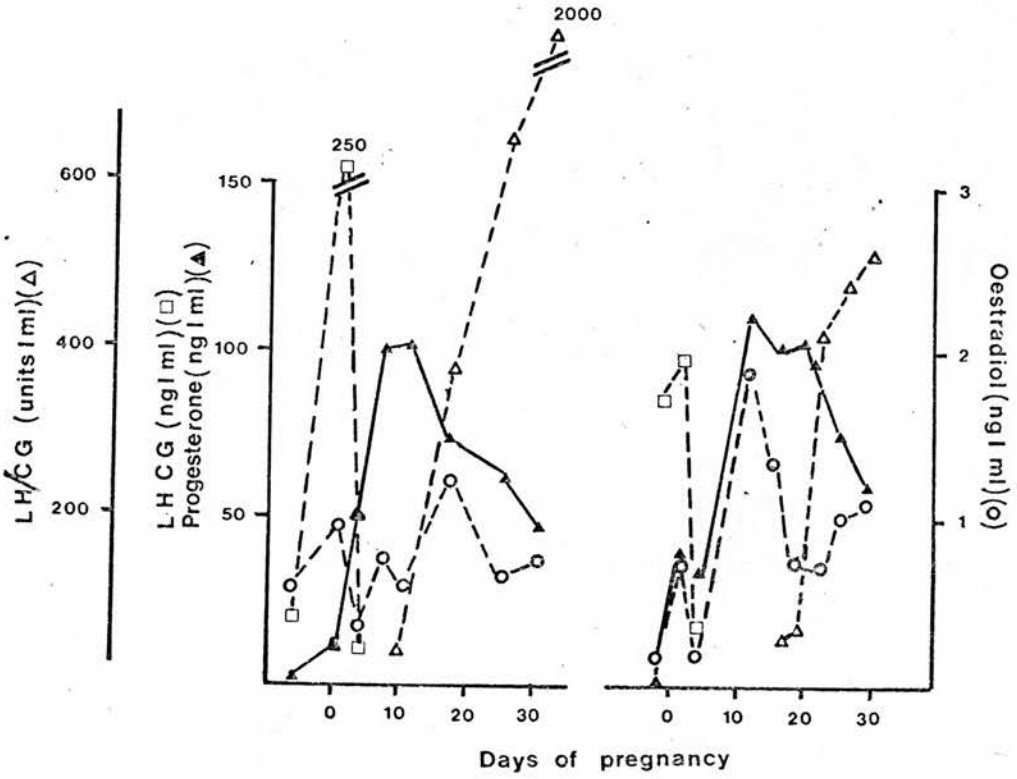
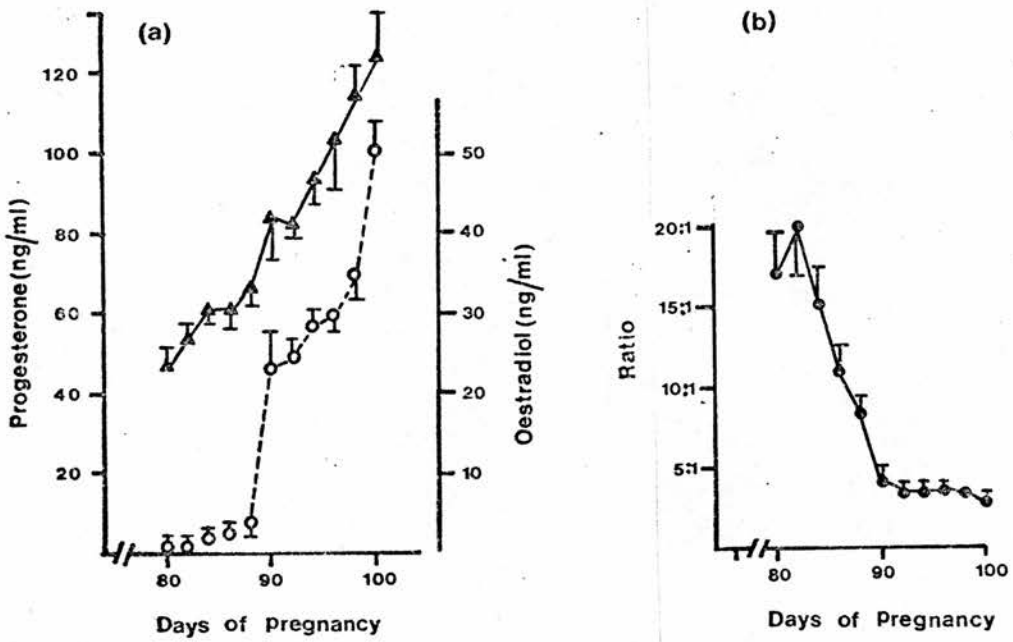


Fig. 4-15(a): The mean (±s.d.) progesterone (▲) and oestradiol (o) levels from 80 to 100 days of pregnancy in the marmoset.

(b): The mean ratio (±s.d.) of progesterone to oestradiol from 80 to 100 days of pregnancy.



oestradiol during the last weeks of pregnancy. Figure 4-15 shows the daily mean values for the last 7 days of pregnancy for 5 animals sampled daily and the hormonal ratio. Figure 4-17 shows the pattern in 2 individuals.

Progesterone values on the day preceding birth were significantly lower ($P<0.001$) and oestradiol values significantly higher ($P<0.01$) than the values 7 days preceding birth. Progesterone values were always lowest on the day preceeding birth and were 20-60% of the maximum values (-7 days) or 40-70% of the preceeding days values ($P<0.001$). The highest oestradiol values were found on the day preceeding birth in only 3 of the 5 animals and they were not significantly higher than the previous days values.

There was a significant decline ($P<0.001$) in the ratio during the last week and in 3 of the 5 animals, progesterone was lower than oestradiol on the day preceeding birth.

(vi) Conception cycle compared to the non-fertile cycle

Figure 4-18 compares the mean progesterone and oestradiol values in the conception cycle with the non-fertile cycle. The mean value for all cycles was used (see section 4.3c). There was no difference in the progesterone values or pattern until 19-20 days after the initial progesterone rise. The decline in the conception cycle was less marked and values did not fall below 20ng/ml. The oestradiol pattern in the conception cycle diverged

Fig. 4-16(a): The daily mean (±s.d.) progesterone (▲) and oestradiol (o) levels during the 7 days preceeding birth in the marmoset.

(b): The mean ratio (±s.d.) between progesterone and oestradiol during the 7 days preceeding birth.

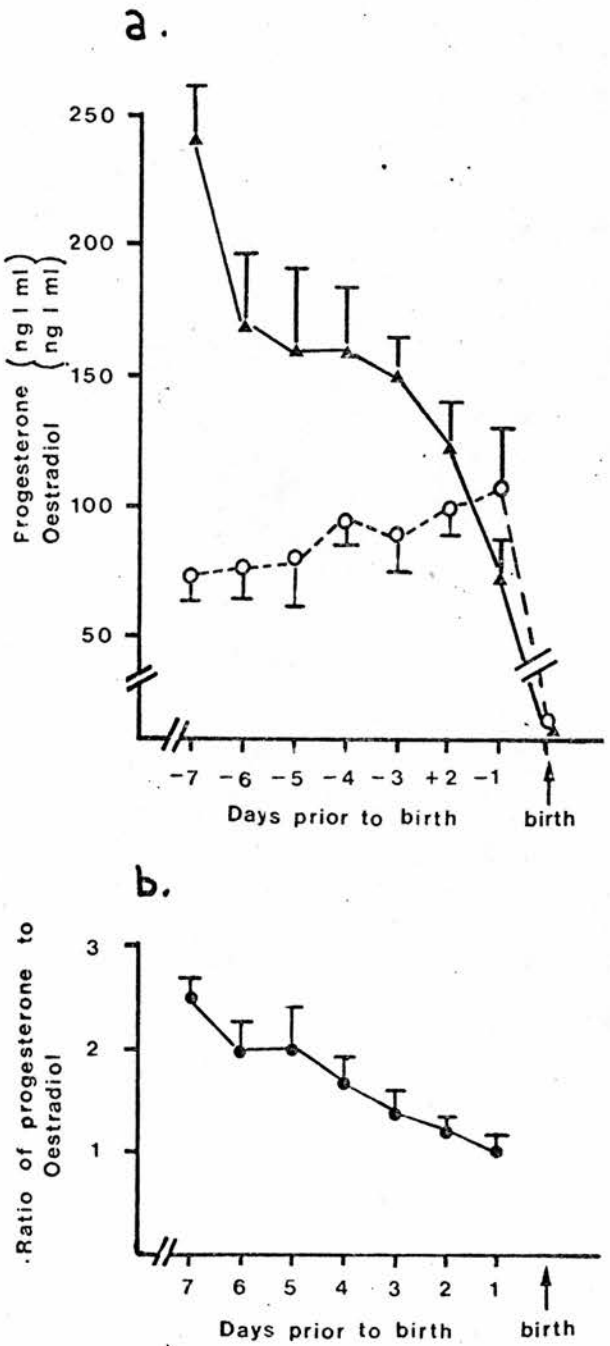


Fig. 4-17: The progesterone (▲) and oestradiol (o) levels in 2 individual marmosets in the 7 days preceeding birth.

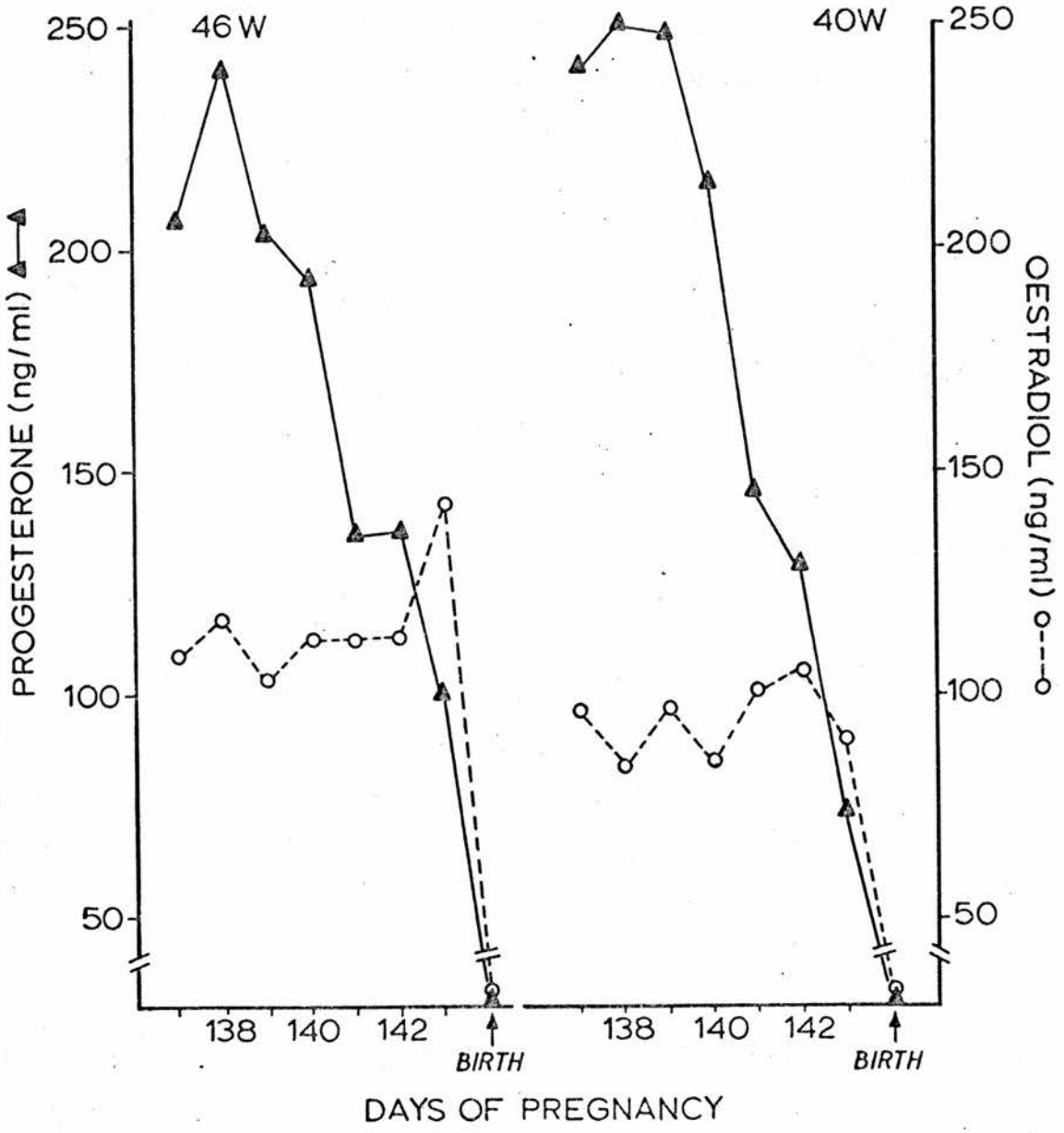
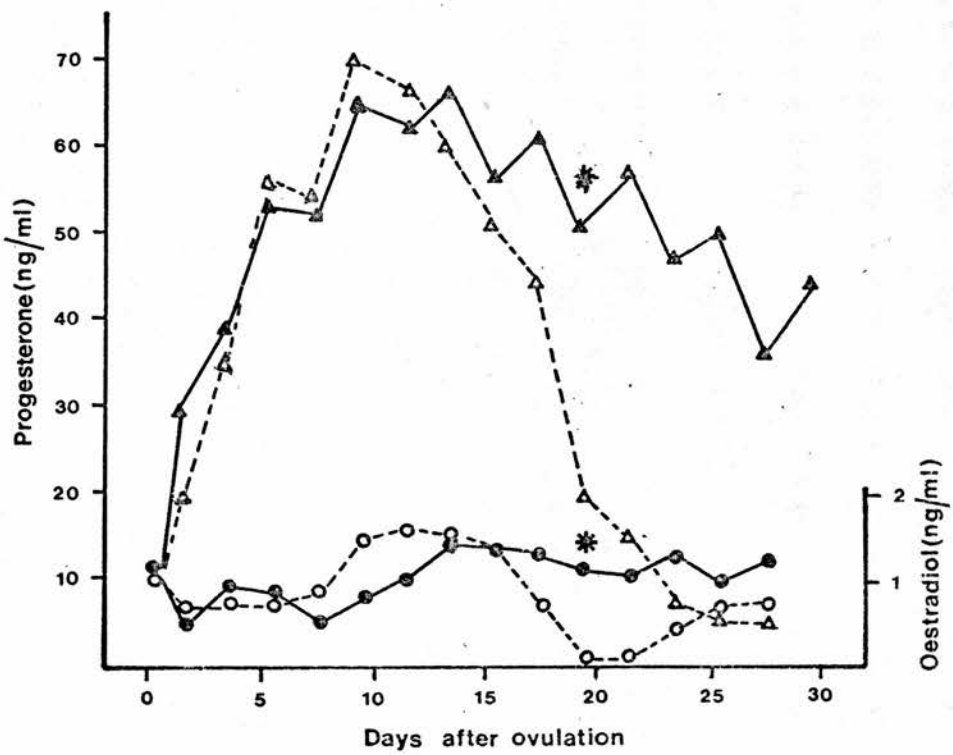


Fig. 4-18: A comparison of the mean progesterone (▲) and oestradiol (●) values in the conception cycle to mean progesterone (Δ) and oestradiol (o) values in the non-fertile cycle. * indicates the first day at which hormonal levels were significantly different between the conception and non-fertile cycle.



from the non-fertile cycle at the same time. Levels fell in the non-fertile cycle but remained high in the conception cycle.

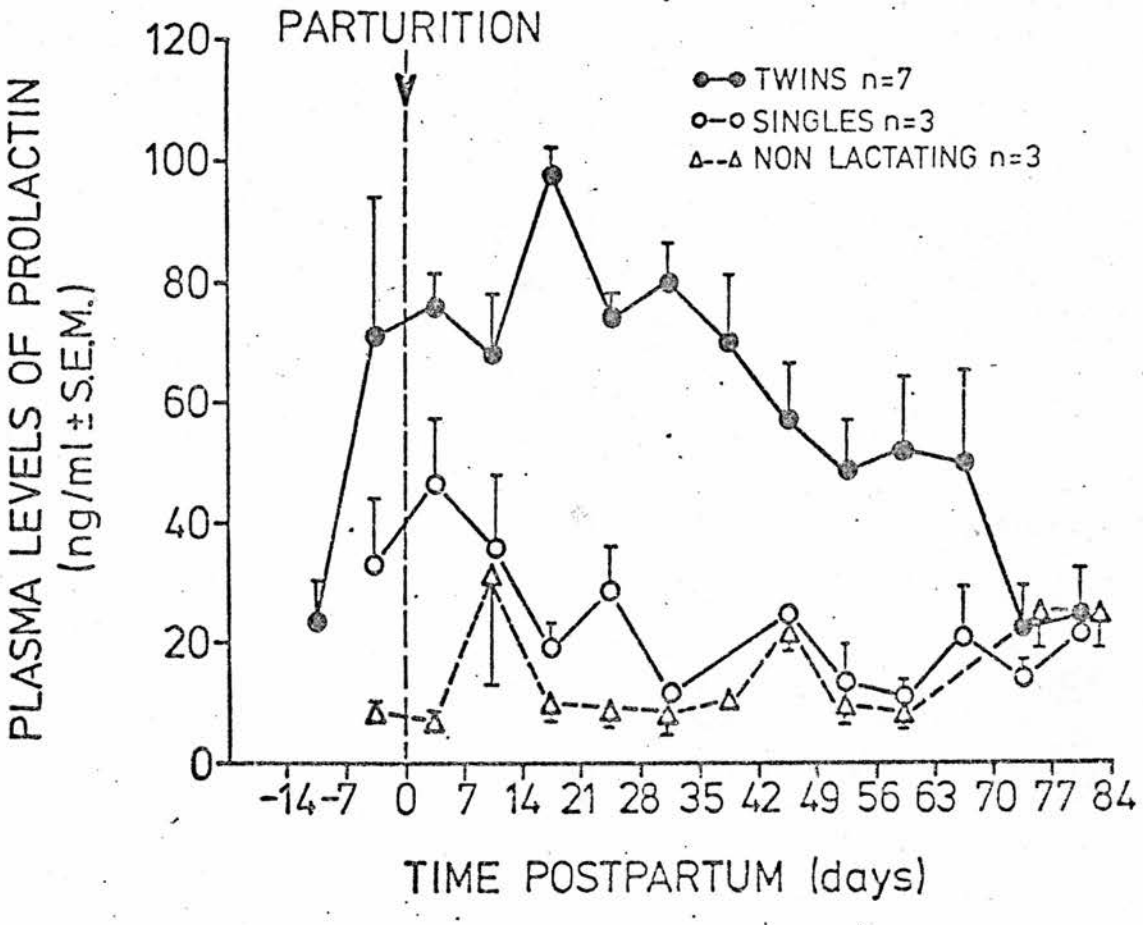
4.3 Prolactin levels during lactation

The changes in levels of prolactin during the last 12 days of pregnancy and during lactation are shown in Figure 4-19. Of the 13 marmosets being bled, 5 delivered triplets, 7 delivered twins and 1 delivered a single infant. Infant death within 5 days of birth resulted in the following groups: non-lactating (N=3), suckling 1 infant (N=3) suckling 2 infants (N=7). There was no relationship between the pre-partum levels of prolactin and the number of young born.

In non-lactating mothers plasma levels of prolactin remained low (<22ng/ml) and were within the normal range for cyclic marmosets (McNeilly et al; 1981) for the duration of the study (up to days 70-77 post-partum). There was an increase in mean plasma levels of prolactin between days 8 and 14, due to transiently elevated prolactin levels observed in one of the three non-lactating mothers.

Plasma levels of prolactin were higher in mothers suckling single infants compared to non-lactating mothers but this only achieved significance ($P<0.05$) immediately after parturition. In contrast plasma levels of prolactin immediately post-partum, and throughout lactation until around day 65 post-partum were significantly higher ($P<0.05$ to $P<0.001$) in mothers suckling twins than both other groups.

Fog. 4-19 : Changes in the plasma levels (mean ±s.e.m.) of prolactin during lactation in marmosets suckling no (Δ; n=3) one (o; n=3) or two (●, n=7) young.



Ovulation occurred in all 13 animals post-partum (Table 4-3). Plasma levels of progesterone and the duration of the luteal phase were not significantly different from those in the normal non-pregnant female.

Twelve animals became pregnant during the observation period, 5 conceiving during the first and the remainder during the second cycle post-partum. The time to first ovulation was not significantly different between the three groups but time of conception was significantly delayed ($P<0.05$) in both groups of lactating mothers compared to the non-lactating group (Table 4-3). Plasma levels of prolactin around conception were also significantly higher ($P<0.05$) in both lactation groups compared to the non-lactating group (Table 4-3, Figure 4-19).

4.4 Discussion

(a) Cycle

Marmosets do not show a conspicious oestrous cycle, characteristic of lower species, or a detectable menstrual cycle, characteristic of higher species. However, like other species, there was a clear hormonal cycle. Progesterone values were several-fold higher than in women (reviewed: Diczfalusy and Landgreen, 1977) and Old World primates (see 4.1 for references). Although there is a general lack of information regarding the circulating levels of ovarian steroids in New World monkeys, it appears that in some species in which levels were also determined, such as the capuchin (Nagle et al, 1979) squirrel (Wolf et al, 1977) and tamarins (Preslock et al, 1973), comparatively

Table 4-3. Days post-partum to first ovulation and conception, the number of cycles to and the plasma levels of prolactin around the time of conception (days -7 to +7) in marmosets suckling no, one or two young.

	Number of young suckled		
	None	One	Two
Days to			
First ovulation	18 ± 4 (3)†	15 ± 1 (3)	29 ± 9 (7)
Conception	28 ± 8 (3)	57 ± 10 (3)	56 ± 11 (6)
No. of females conceiving in successive cycles post-partum			
1st	2	0	3
2nd	1	3	3
Other	-	-	1*
Plasma prolactin at conception (ng/ml)	8 ± 1 (5)	23 ± 8 (4)	40 ± 18 (6)

Number of observations in parenthesis

† Mean ± s.e.m.

* Conception did not occur during the observation period.

high levels of circulating progesterone were also found. Although it may be postulated that this denotes a significant difference between New and Old World monkeys, the physiological significance of these differences remains to be elucidated.

There was a definite luteal phase oestradiol rise and in this respect the marmoset more resembles the human and chimpanzee than the rhesus and baboon (section 4-1 for references). The hormonal patterns around ovulation also show general similarities to the human but the exact relationship between progesterone, oestradiol, LH/CG and ovulation has not been determined by direct ovarian observations or by daily sampling regimes with measurement of all 3 hormones.

The follicular phase of the cycle was relatively short and the control of follicular development in this species has yet to be studied to determine its similarity to the human (Baird, Baker, McNatty and Neal, 1975). The luteal phase of the cycle was not prolonged by hysterectomy (Hearn, 1978) implying that the corpus luteum was not under luteolytic control as in the sheep, guinea pig, cow and other species (reviews: Short, 1969; Heap and Perry, 1974).

No definite estimation of cycle length was obtained from this study. Although the duration of the sampling should have resulted in at least 6 complete cycles based on previous marmoset studies (Hearn and Lunn, 1975), only 2 complete and 1 incomplete cycles were obtained. Fewer cycles than expected were obtained due to the

acyclicity of 2 marmosets and to the relatively longer luteal phase found in the present study. Urinary oestrogen data (Lunn, 1981) and subsequent studies on serum hormonal levels (McNeilly et al, 1981; Harding, pers'l commun) support a cycle length similar to that reported in this thesis.

The duration of and the hormonal profiles in several cycles obtained from females paired with fertile males was similar to the non-fertile cycle and there was no rise in LH/CG in the luteal phase as found in the conception cycles. It was felt justified to calculate mean cycle values including these animals for comparison with the conception cycles, although conception and early embryonic loss could not be definitely excluded. A high percentage of early embryonic loss has been reported for the human (James, 1970; Cutright, 1975; Karow and Gentry, 1976) with either no change or a lengthening of only a few days in the cycle length. However, in the human, conception and subsequent embryonic loss were indicated by rise and fall in LH/CG values during the luteal phase. This was also found in some marmosets with apparently long luteal phase lengths of more than 25 days (unpubl'd abservations) and these animals were not included.

(b) Pregnancy diagnosis and the dating of pregnancy

In the absence of a conspicuous menstrual or oestrus cycle, clearcut cyclical vaginal cytology (Hampton and Hampton, 1975; Hearn and Renfree, 1975) or mating behaviour (Hearn, 1978), the most accurate means of detecting

ovulation and dating subsequent pregnancies is by hormonal measurement. Progesterone levels were used to indicate ovulation and pregnancy was confirmed 3-4 weeks later by continued high progesterone levels and by abdominal palpation. This method proved adequate for the investigations in this thesis as several accurately dated pregnancies were obtained by simply monitoring animals following birth or abortion. Since a high percentage of animals ovulate and become pregnant following birth, blood sampling needs to be continued only for a short time. The method is not too time consuming in this species as blood sampling is easily manageable by 1 individual and the hormone assays are simple and quick. The disadvantage is that only a limited number of animals may give birth during any 1 time period and the method is more time consuming than the methods used in other species in which there is physical evidence of cyclicity. A disadvantage to studies in early pregnancy is that there is as yet no means available for confirming pregnancy until nearly 4 weeks after ovulation. Although LH/CG may rise during the 3rd week, the assay takes 5 days to complete. The mean progesterone and oestradiol profile diverge near the end of the 3rd week in the fertile and non-fertile cycle but considerable individual variation in the hormonal profiles would not allow definite confirmation of pregnancy at this time. Pregnancy diagnosis kits have not proved successful prior to 4 weeks of pregnancy (Poswillo et al, 1972a; Mitchell and Jones, 1975; Hodgen et al, 1976; Hobson et al, 1977).

The best and quickest means of estimating the stage of gestation in animals already pregnant is by abdom-

inal palpation. This could confirm pregnancy at about 4 weeks after ovulation and was thereafter reasonably accurate particularly if done weekly by the same individual. Comparison of the abdominal palpation with the uterine measurement taken at operation showed that the values obtained were generally similar. An additional guide to the stage of pregnancy during later pregnancy was measurement of the fetal heads and hormonally, by the progesterone and oestradiol rise which occurred at 90 days. Measurement of oestradiol in particular may aid in dating pregnancies, as there was a consistent interanimal relationship between the stage of gestation and the initiation of a marked increase in concentration.

(c) Quantitative hormonal comparisons during pregnancy.

There are large quantitative differences and differing relative amounts of plasma steroids secreted during pregnancy in various primate species (Table 4-4). As in the rhesus monkey and chimpanzee, progesterone was quantitatively the most important steroid hormone in marmoset plasma and progesterone levels at the end of gestation in the marmoset resembled those in women more than those in the chimpanzee or rhesus monkey. Relatively large amounts of oestrogens were found in the marmoset compared to other primate species and compared to the progesterone value in the marmoset plasma. The cause or physiological significance of these exceptionally high oestrogen values is not known. Free oestriol, which was also found in large amounts in plasma during human pregnancy (Tulchinsky et al, 1972) was not measurable in marmoset plasma (<0.125ng/ml).

Table 4-4. The mean or range of plasma steroid hormone levels (ng/ml) during the last week of pregnancy in some primates.

Species	Progesterone	Oestradiol	Oestrone	Androstenedione	Testosterone	Reference
Man	180	17	7	5	2	De Hertogh et al (1975), Tulchinsky et al (1972), Gandy (1971).
Chimpanzee	49-120	5-8	1.8-3.0	-	-	Reyes et al (1975),
Rhesus Monkey	4-12	0.700	0.370	1.4-2.0	0.38-0.42	Challis et al (1974, 1975), Atkinson et al (1975).
Marmoset	229*	91	23	7	1	Present study.

* Maximum progesterone level at Week 18.

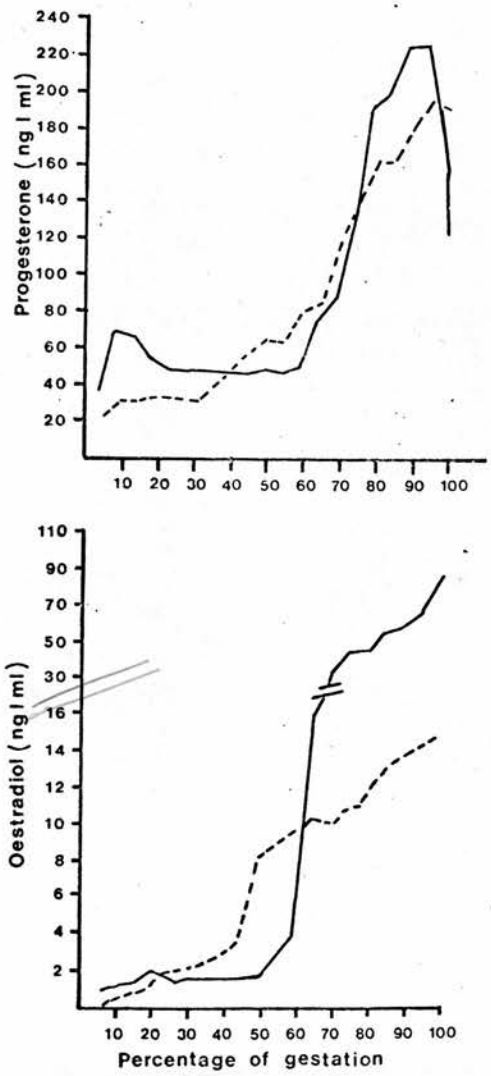
(Chambers, unpubl'd observations). Whether this is due, as in the rhesus, to the failure of the fetal adrenal and liver to produce sufficient amounts of 16-hydroxylated intermediates for oestriol formation by the placenta (Heinrichs and Colas, 1970) is not known. Androgen values were similar to those in the human, but are the only 2 hormones in marmoset plasma for which the term or near-term values do not reflect the maximum values seen during pregnancy.

(d) Hormonal profiles during pregnancy

The hormonal profiles of other primate species were reviewed in Chapter 1 (see for references). The present study showed that the hormonal profile of progesterone and oestradiol resembled that in women (Figure 4-20) in whom luteal phase levels were maintained for the first quarter of gestation followed by a sustained gradual increase (Tulchinsky et al, 1972; Tulchinsky and Hobel, 1973). In the marmoset, progesterone levels, generally slightly lower than luteal phase levels, were also maintained; but for a relatively longer time. The sustained increase in progesterone did not occur until nearly 2/3rds of pregnancy was completed. The magnitude of the increase was less than in women, due to the comparatively high luteal phase values.

In both the human and marmoset, oestradiol gradually increased during the period of relatively stable progesterone values followed by a greater increase coincident with the greater increase in progesterone. Although the overall magnitude of the increase for oestradiol

Fig. 4-20: The mean progesterone and oestradiol profile in the human (---) and marmoset (—) during pregnancy. The human data was taken from Tulchinsky et al 1972 and Tulchinsky and Hobel, 1972.



was similar for the human and marmoset, the increase during the first part of gestation was much more gradual and the increase that was conincident with the progesterone increase (90 days) was much steeper in the marmoset.

The LH/CG profile resembles that found in the human in several respects. The life of the corpus luteum is extended coincident with rising gonadotrophin levels, thus distinguishing the fertile and non-fertile cycle. Subsequently corpus luteum function declines (See Chapt. 5) simultaneous with the rapidly increasing CG levels. Although peak CG levels were found relatively later in gestation in the marmoset (40-50% of pregnancy completed) than in the human ($\sim$ 20%), the temporal relationship of CG to progesterone and oestradiol was similar. Peak values are found during the period when peripheral progesterone levels are stable and oestradiol levels are gradually increasing. In the marmoset, the decline in LH/CG may be more advanced when the peripheral progesterone and oestradiol levels increase.

It seems that although there are some similar

hormonal patterns overall and interhormonal temporal relationships between the marmoset and the human, the profiles found in the human during approximately the first trimester of pregnancy are lengthened in the marmoset to nearly two-thirds of pregnancy. There may be an overall slower development of the events that occur during the early part of gestation. This will be discussed further with respect to ovarian and placental function, fetal hormones and development in Chapters 5, 6 and 7.

Although like the human (Tulchinsky et al, 1972) mean oestrone values increased during pregnancy, there was considerable interanimal variation in the oestrone profiles. Some individuals showed no increase in levels and there was no obvious explanation for the differences in profiles. The animals studied did not have either unusual oestradiol values or profiles, or oestradiol: oestrone ratios. The difference between individuals also could not be accounted for by the number of young born or the outcome of the pregnancy. Perhaps studies on the dynamics of oestrone production and metabolism will illuminate the reasons for interanimal variation.

Oestrone values in the marmoset were relatively high for more than the first half of pregnancy and they far exceeded oestradiol values. Research has mainly focused on oestradiol as it is the more potent oestrogen and for other primates it is the major plasma oestrogen (see Chapter 1 for references). In the marmoset, oestrone is the major plasma oestrogen during the first part of pregnancy due to

its greater secretion by the corpus luteum (see Chapter 5). This indicates that it is not merely an inter-species difference in peripheral hormonal inter-conversions or in metabolic clearance rates, but a difference in secretion by the corpus luteum (human :Baird and Fraser, 1974). Despite its lower levels compared to oestrone, oestradiol probably still has a physiological significance similar to the human. Its profile shows marked similarities around ovulation (Hodges, 1977), during the cycle (Hearn and Lunn, 1975) and in early pregnancy (Chambers and Hearn, 1979). A negative and positive feedback effect of oestradiol on LH was also demonstrated for the marmoset (Hodges, 1978). The physiological significance of oestrone has not been investigated.

Even in species in which oestrone is the major urinary oestrogen (e.g. baboon, Townsley, 1972) oestradiol-17 is still the major plasma oestrogen. It seems likely that there are major differences between the marmoset and human in their metabolism of both oestrogen and progesterone. In the marmoset oestradiol-17β and 6β-hydroxypregnanolone are the major metabolites (Schackleton, 1974; Lunn, 1978) whereas in woman, pregnanediol and oestriol are the major metabolites (Bell and Lorraine, 1971).

Like the human (Chapter 1 for references), androgen levels increased during pregnancy but in the marmoset the increase was not sustained. In fact, the androgen profile in the marmoset was similar to the LH/CG profile with maximum levels around 7-10 weeks. The factors responsible for, or the physiological significance of, increased androgen levels during pregnancy are not definitely

known for other species (see Chapter 1); however, maternal as well as fetal andogen precursors are utilised for placental oestrogen production (Siiteri and MacDonald, 1966). Although androgen precursors may also be important for placental oestrogen production in the marmoset (Ryan et al, 1961; Ryan and Hopper, 1974) the relative contribution of maternal and fetal androgen precursors to oestrogen production is not known. Whether there is a relationship between the declining maternal androgen levels and the increasing oestrogen levels during the latter part of pregnancy must await further studies. It would be interesting to compare the effects of dexamethasone administration on androgen and oestrogen levels in this species with the effects found in the human (Ohrlander et al, 1977) and rhesus (Challis et al, 1974, 1975, 1977b; Bosu et al, 1974b).

Unlike the human (Kletzky et al, 1980), there was no overall increase in prolactin levels during pregnancy and therefore prolactin levels did not parallel the oestradiol or progesterone levels. A rise in prolactin at the end of gestation, when maximum oestradiol levels were found, was no higher than a transitory rise at 10-12 weeks of gestation, which was prior to the steep increase of oestradiol at 13 weeks.

There was no increase in prolactin levels except possibly near the end of pregnancy in the rhesus, but this correlated with the lack of a marked increase in oestrogen or progesterone also found (Weiss et al, 1976). It appears

that a relationship between prolactin and either progesterone or oestradiol is not found in the marmoset.

(e) Early pregnancy

Like other primate species so far studied, there was an extension of luteal phase progesterone values into early pregnancy concomitant with rising peripheral LH/CG levels. A more sensitive LH/CG assay may be necessary to definitely establish the timing of the initial rise in gonadotrophin and its relationship to the progesterone profile in the conception cycle. However, there was no difference in the progesterone profile between the conception cycle and the non-fertile cycle until nearly 3 weeks after ovulation when levels in the fertile cycle were declining rapidly. Levels were also often declining in the conception cycle although the decline was less steep. Unlike the rhesus, there was no evidence of a progesterone 'surge' associated with rescue of the corpus luteum (Neill et al, 1969; Hodgen et al, 1972) and unlike the human, progesterone did not continue to increase during the initial period of corpus luteum 'rescue' (Tulchinsky and Hobel, 1973). The relationship of the progesterone profile to corpus luteum function will be discussed further in Chapter 5.

Hormonal values fluctuated considerably during the first part of pregnancy. Detailed analysis in the rhesus showed progesterone nadirs were associated with oestradiol peaks and definitive peaks of progesterone were associated with specific stages of gestation (Hodgen et al,

1972; Atkinson et al, 1975 and others). However, no such temporal or interhormonal relationships were found in this study in the marmoset.

Whereas the mean trends were fairly obvious, serial samples from individual animals sometimes showed considerable variability from these general trends and levels. This factor must be borne in mind by an investigator when data is considered for a single individual.

(f) Late pregnancy

The marmoset is the first primate species so far studied in which there is a significant decline in plasma progesterone during the last weeks of pregnancy. In this respect it more resembles the sheep, cow, pig, goat and rat (reviewed: Thorburn et al, 1977). In contrast, oestradiol trends are similar to those in women (Liggins, Forster, Grieves and Schwartz, 1977; Fuchs, 1977) rhesus monkeys (Challis et al, 1977b) and chimpanzees (Reyes et al, 1975; reviewed: Lanman, 1977) in which the maximum concentrations are found in the last week of pregnancy and there is no pronounced pre-partum increase as is found in the sheep (Thorburn et al, 1977). Progesterone often declined to values lower than oestradiol on the day preceding birth and the ratio between these 2 hormones was lower than for any other primate species so far studied. The significance of the changing peripheral plasma hormonal levels and ratios in relation to parturition is not known in the marmoset as there have been no investigations in this species on the factors influencing these late pregnancy hormonal changes,

or on their significance for the timing of parturition (see also Chapter 5.4). It is unknown whether the abolition of the pre-partum decline in progesterone would delay parturition in the marmoset. In the sheep, a massive dose of progesterone, at least twice the daily production, was necessary to delay parturition (Hindson, Schofield and Ward, 1969; Liggins, Fairclough, Grieves, Kendall and Knox, 1973).

(g) Prolactin levels during lactation and the occurrence of ovulation.

This study clearly showed that in the marmoset high levels of prolactin and a maintained suckling stimulus did not inhibit the resumption of ovarian acitivity and the return of fertility post-partum. Lactation in the marmoset was associated with elevated plasma levels of prolactin similar to those in the rhesus monkey (Butler et al, 1975) and in women (Rolland, Lequin, Schellekens and de Jong, 1975). In contrast to these primates, there was no delay in the return to ovulation post-partum. Similarily, the luteal phase associated with the first ovulation was of normal duration and progesterone levels were normal. The first post-partum cycle was also capable of maintaining a pregnancy unlike breast feeding women, where luteal function after first ovulation is usually inadequate (McNeilly, 1979, 1980; Duchen and McNeilly, 1980; McNeilly, Howie and Houston, 1980).

The time to conception was delayed in lactating mothers compared to non-lactating mothers in this section of the study. However, a delay in the time to conception related to suckling is not a consistent finding as 3

marmosets suckling twins also conceived at the first ovulatory cycle. Also, in a previous study (section 4.3b) the majority of the animals who conceived at the first ovulatory cycle were lactating. Birth interval data for the whole colony confirms that lactation *per se* does not influence the time of conception post-partum (Lunn and McNeilly, 1981). Whether *C. jacchus* differs from other callitrichid monkeys is uncertain, since it has been reported that in the genus *Saguinus*, mating and ovulation do not occur for 2½ months post-partum (Wendt, 1964) and lactation delays subsequent pregnancy (Wolf, Ogden, Deinhardt, Fisher and Deinhart, 1972; P. Abbot and C. Snowden, pers'l commun.).

4.5 Chapter Summary

1) The gestation length was 144 ± 2 (s.d., N=9) days.

2) Ovulation occurred 10.5 ± 0.7 (s.d., N=23) days following birth.

3) Lactation did not interfere with the return to fertility although prolactin levels were significantly elevated in mothers suckling twins.

4) The stage of gestation could be assessed by a) the post-ovulatory progesterone rise (timed pregnancies) b) abdominal palpation of the uterine diameter after the 4th week of pregnancy and of the fetal heads after the 15th week, and c) by the oestradiol and progesterone rise at 88 to 92 days of pregnancy.

5) Corpus lutea were generally distinguishable up to day 90, were distributed either on 1 or both ovaries, and were generally correlated with the number of fetuses present.

6) The hormonal profiles of the 5 steroid hormones, LH/CG and prolactin were shown throughout pregnancy. Both mean and individual trends were given. The conception cycle and the first 70 days of pregnancy, 80 to 100 days and the last week of pregnancy were studied in detail for some of the hormones. The mean profiles were:

a. Progesterone declined from week 3-5, remained stable until week 13, increased rapidly at week 13 and declined significantly in the last 1½ weeks of pregnancy.

b. Oestradiol gradually increased until week 13.

Coincident with progesterone, levels increased rapidly at week 13. Unlike progesterone, maximum levels were found in the last week of pregnancy.

c. Oestrone levels increased after ovulation and at 3-4 weeks. There was a sustained increase at week 16.

d. Androstenedione and testosterone levels increased until week 8 and thereafter declined.

e. LH/CG increased during the 2nd-3rd week to maximum levels at week 8 and thereafter declined. The highest levels were found during the period of relatively stable progesterone levels.

f. Prolactin levels declined between week 10 and 19, and increased during the last week of pregnancy.

7) The conception cycle and the nonfertile cycle diverged at day 19 for the progesterone and oestradiol profiles.

8) The progesterone to oestradiol ratio declined during the first 14 weeks of gestation, remained relatively stable until week 18, and then declined during the last 2½ weeks of pregnancy.

The oestradiol to oestrone ratio increased rapidly between the 12th and 15th week of pregnancy. Oestrone was the major plasma oestrogen for the first 12 weeks of pregnancy and oestradiol was the major plasma oestrogen after week 13.

The androstenedione to testosteone ratio gradually increased with advancing gestation.

CHAPTER 5 : OVARIAN AND PLACENTAL FUNCTION

5.1 Introduction

Hormonal trends in the human and other sub-human primates, primarily the rhesus, have been related to ovarian and placental function in these species. This was reviewed in Chapter 1. There have been no previous reports on ovarian and placental function in the marmoset.

There are some similarities between the marmoset (Chap. 4) and the human in peripheral hormonal trends, an obvious one being the sustained increase in progesterone and oestradiol during the latter half of pregnancy. However, there were also major differences. The increase in progesterone and oestradiol did not occur until relatively late in gestation and it was suggested that there was a slower development of events during the first part of pregnancy (see Chapter 4, discussion). Hearn (1978) has shown that the luteo-placental shift may not occur until nearly one-third of pregnancy is completed, which is considerably later than in other species. Oestrogen levels were also comparatively high in this species and the oestradiol to oestrone ratio was unusual among primates. The androgen profile was also at variance with that reported for the human (human references : Chapter 1).

The studies in this chapter investigated the changes in ovarian and placental function throughout pregnancy. Is the ovarian contribution to hormonal levels important for longer in pregnancy than in other species and when does the placenta assume a major role in hormone production? What

is the relationship between ovarian - placental function? How does ovarian - placental function relate to the maternal and fetal profiles? Also, what are the hormonal levels andratios in the utero-ovarian vein and placental tissue and how do they compare with each other and with the stage of gestation? Can an *in vitro* system be established to look at changes in placental hormonal secretion with advancing gestation and be used to investigate the regulation of the hormonal secretion?

In order to fulfill these aims, techniques were chosen that allowed the maximum integration of data, minimised the occurrence of abortion during the experiment, did not jeopardise further colony breeding and considered the relatively small size of the marmoset. Maternal and utero-ovarian vein blood, placental tissue and fetal samples (Chapters 6 and 7) were obtained at laparotomy or hysterotomy throughout pregnancy utilising primarily accurately dated pregnancies. The multiple sampling sites and the measurement of several hormones for each sample allowed inter-compartmental hormonal comparisons within each pregnancy.

The results section is divided into 3 parts. The first part reports on the *in vivo* assessment of gestational changes in ovarian and placental function by hormonal measurements in utero-ovarian plasma obtained throughout pregnancy. The hormonal values were related to the presence or absence of corpus luteum and of the feto-placental unit and were compared with the maternal peripheral plasma hormonal value.

The second part reports on the gestational changes in the hormonal concentrations and total content of placental tissue. Placental hormonal values and ratios were established and then compared with those in the maternal, utero-ovarian and umbilical vein plasma. Two placental discs were always found, and the hormonal concentrations were compared between the discs and related to the fetal attachments and sex.

Part 3 reports on the establishment of organ cultures of placental tissue, the secretion of progesterone and LH/CG by placental tissue maintained in organ culture and the influence of various factors on progesterone and LH/CG secretion. The in vitro secretion was related to hormonal trends in the maternal and utero-ovarian vein plasma and to the placental content.

5.2 Procedures

(a) Part I: Utero-ovarian vein samples

The sampling regimes have been outlined in Chapter 2.7 and the procedure for obtaining utero-ovarian vein (UOV) samples in Chapter 2.6. Progesterone, oestradiol and oestrone were measured on nearly all the samples. LH/CG was measured in samples when there was sufficient plasma.

(b) Part II: Placental hormonal content

Placental tissue was obtained for hormonal content measurements between days 40 and 140 as described in Chapter 2.8. Progesterone, oestradiol, oestrone,

testosterone, androstenedione were measured as described in Chapter 3.2k and LH/CG as described in Chapter 3.3.

Except at day 40, each placental disc was assessed separately. After day 60 at least 3 homogenates were prepared from each disc. The mean value from each homogenate was used to calculate the mean concentration (ng/g) per placental disc or for 2 placental discs. The total content was calculated by multiplying the cencentration by the placental weight (Chapter 7) and is expressed as ng/placenta.

(c) Part III: Placental organ cultures

Placental tissue was obtained for culture following hysterotomies between 30 and 140 days of pregnancy as described in Chapter 2.6 and 2.7.

The pieces of the placental disc for culture were weighed. The weights used ranged from 0.15 to 0.25 grams on tissue obtained after day 50 of pregnancy. Due to the small size of the placenta at 30 (0.03 grams) and 40 (0.20 grams) days, pieces for culture weighed less than 0.05 grams. The placental tissue was gently eased apart and rinsed with saline.

The prepared tissue was cultured essentially as described by Baker and Neal (1969). The tissue was placed on a stainless steel grid covered by a piece of lens paper, in a 3mm diameter petri dish (Figure 5-1) containing 2ml of Eagle's minimum essential medium with Earle's salts, buffered with 20mm HEPES buffer, and was supplemented with newborn calf serum (20% v/v), L-glutamin (2mM/L) and anti-

biotics (Amphotericin B, 1.5ug/ml; Kanamycin, 30ug/ml). All the media used were obtained from Flow Laboratories, Irvine, U.K.

Racks of five of these petri dishes were placed in modified 'Kilner' preserving jars (Figure 5-1) which were gassed at 0.704kg/cm^2 with 5% CO_2, 38% N_2 and 57% O_2, and then incubated at 37°C.

The average time between removal of the feto-placental unit and the placental tissue being placed in the incubator was approximately 3-4 hours. Every 24 hours the culture medium was removed and replaced with fresh medium. The medium was stored at -20°C for radioimmunoassay.

The histology of the tissue following culture was examined by Dr. O and Professor T.G. Baker, Department of Obstetrics and Gynaecology, and in most instances the tissue appeared viable with little evidence of cell death. Any cultures that were not viable, or which had been contaminated, were not included in the results.

Additions

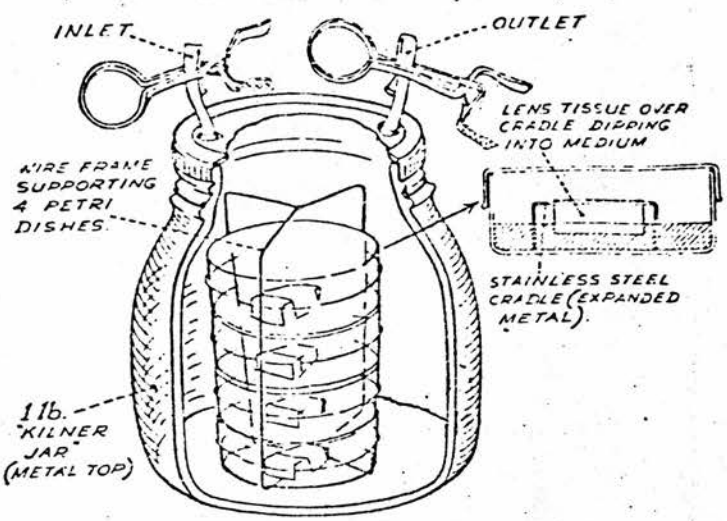
The following preparations were added to the cultures. All preparations were made up in culture medium.

a) Progesterone (Sigma). Concentrations of 50 and 200ng/0.3ml were prepared. 0.3ml was added to culture to give a final concentration of 25 and 100ng/ml.

b) Pregnenolone (Sigma). Concentrations of 70ng/0.3ml were prepared. 0.3ml was added to culture to give a final concentration of 35ng/ml.

c) Cholesterol (Sigma). Concentrations of

Fig. 5-1: Diagrammatic representation of the "Kilner jar" culture apparatus. Note that 5 petri dishes could be supported within one jar.



500ng/0.3ml were prepared. 0.3ml was added to cultures to give a final concentration of 250ng/ml

d) hCG (Sigma). Ten fold dilutions ranging from 0.01 i.u./0.3ml to 1,000 i.u./0.3ml were prepared 0.3ml was added to cultures to give final concentrations of 0.005-500i.u./ml.

e) Antiserum to hCG and serum. 0.01 to 0.1ml of plasma from non-pregnant marmosets, which were actively immunised against hCG and had high circulating levels of antibodies, (Hearn, 1978; Hearn et al, 1978) was added. Similar quantities of plasma from non-pregnant animals was added to cultures as controls.

f) Fetal gonads and adrenals. A section (unweighed) of the fetal gonad (ovaries and testes) and the fetal adrenal were placed next to the placental tissue. Sections were also cultured without placental tissue using the same methodology.

5.3 Results

Part I: Hormonal values in the utero-ovarian vein

(a) Follicular phase

UOV samples were taken from 2 animals 3-4 days after giving birth and 6 days prior to ovulation. Progesterone was less than the assay sensitivity (2-7ng/ml) in both the UOV and MPV (maternal peripheral vein). MPV oestradiol values were 1.5 and 1.1ng/ml. UOV oestradiol values were 1.8 and 0.4ng/ml. MPV oestrone values were 1.2ng/ml and UOV values were 6.7 and 1ng/ml. Large developing follicles were apparent in

the animal with higher UOV oestrogen values.

(b) General trends during pregnancy

The UOV concentrations are reported for each hormone as a total value for the right and left UOV. The corresponding MPV value for each pregnancy is also given. For all hormones the concentrations and changes in the MPV were similar to those reported in Chapter 4.

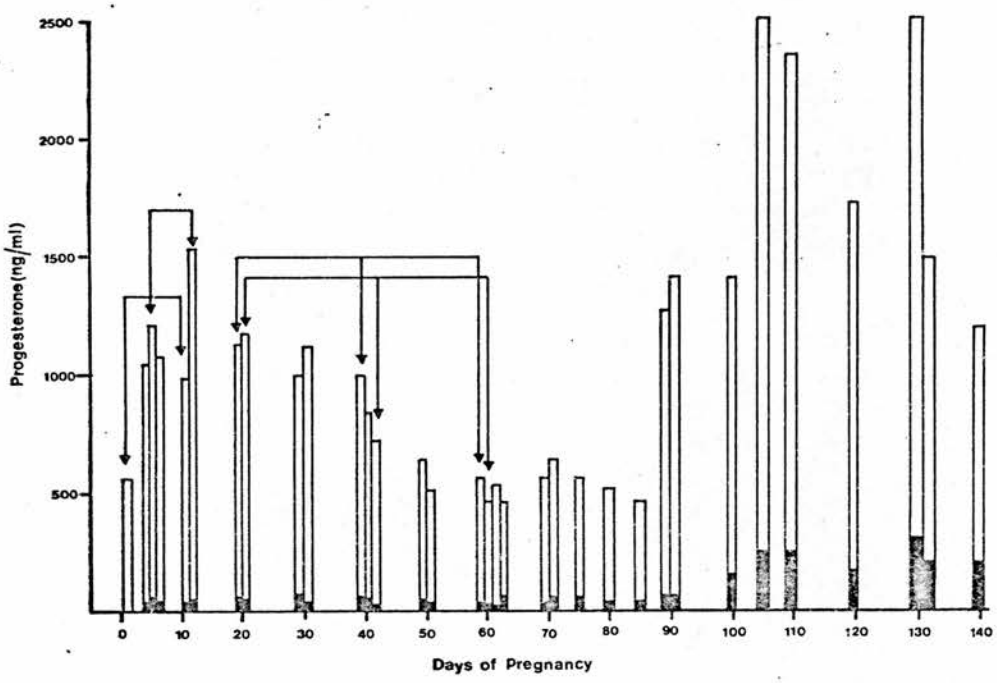
(i) Progesterone

The UOV and MPV progesterone concentrations are shown in Figure 5-2. Just after ovulation (day 1), there was an intermediate rise in progesterone followed by luteal phase levels by the 5th to 10th day of the conception cycle. This was clearly shown for 2 individuals. Luteal phase levels subsequently declined in the UOV until day 60. Little change occured between days 60 and 80. The mean progesterone level in the UOV between days 60 and 80 was approximately 50% of the luteal phase level and was significantly lower ($P<0.001$, t-test, $n=17$).

These trends in progesterone values in the UOV during early pregnancy were confirmed in UOV samples taken at serial laparotomies at 20, 40 and 60 days of gestation in 2 animals. By day 60, progesterone values in the UOV were less than 50% of the value at day 20 and in 1 of the animals, the levels in the peripheral vein declined similarly.

There was a 3 fold increase between 80 and 90 days of gestation. Maximum values were reached

Fig. 5-2: The sum of the progesterone values (ng/ml) in the right and left utero-ovarian vein (∏) and the progesterone value in the maternal peripheral plasma (■) throughout pregnancy. The arrows link pregnancies from which serial samples were taken.



between 105 and 130 days of gestation. These maximum levels were more than 2 times the mean luteal phase level and 4 times the mean 60 to 80 day level. Maximum levels were not correlated with either placental size or the number of fetuses present (Chapter 7). UOV progesterone values declined in the last 10 days of gestation and at day 140 were similar to those found at 90 to 100 days.

(ii) Oestradiol-17β

The UOV and MPV oestradiol concentrations are shown in Figure 5-3. There was no consistent change in values for the first 50 days of pregnancy. There was a gradual increase between days 50-85 followed by approximately a 10 fold increase at day 90. Thereafter, levels remained elevated and were highest at 140 days. The 140 day level was about 7 times the 90 day level.

(iii) Oestrone

The MPV and UOV oestrone concentrations are shown in Figure 5-4. Immediately following ovulation, oestrone levels were 9ng/ml. There was no significant change in values during the first 80 days of pregnancy. Mean oestrone values after day 90 of gestation were significantly higher than those prior to day 90 ($P < 0.001$, t-test, n=31) although there was some overlap between the 2 periods.

(iv) LH/CG

LH/CG was measured in UOV samples from 6 animals between days 10 and 100. UOV values were similar to the MPV values and there was no significant difference between the UOV and MPV levels (paired t-test). Values

Fig. 5-3: The sum of the oestradiol values (ng/ml) in the right and left utero-ovarian vein (▯) and the oestradiol value in the maternal peripheral plasma (▮) throughout pregnancy. The arrows link pregnancies from which serial samples were taken.

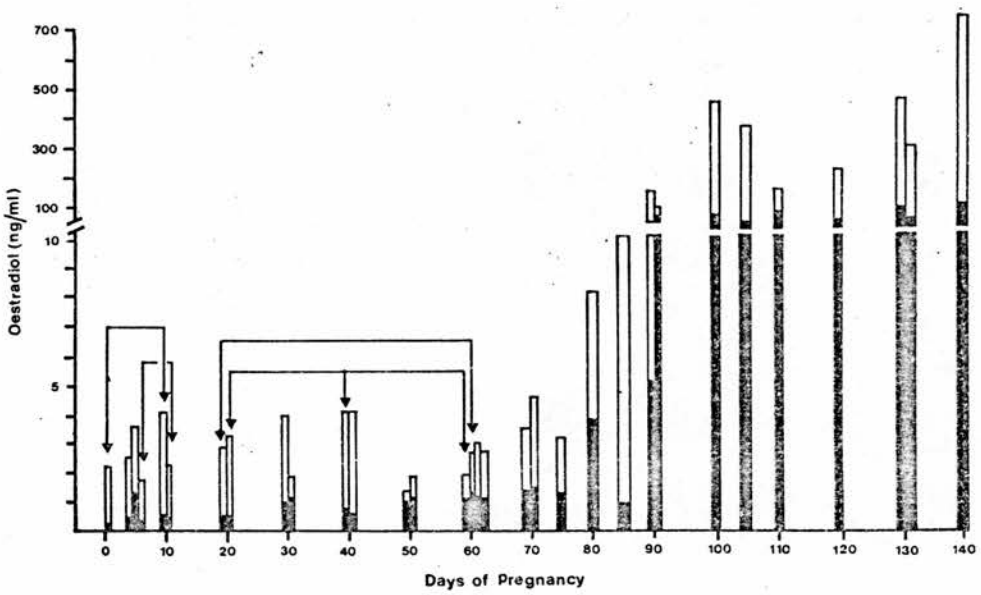
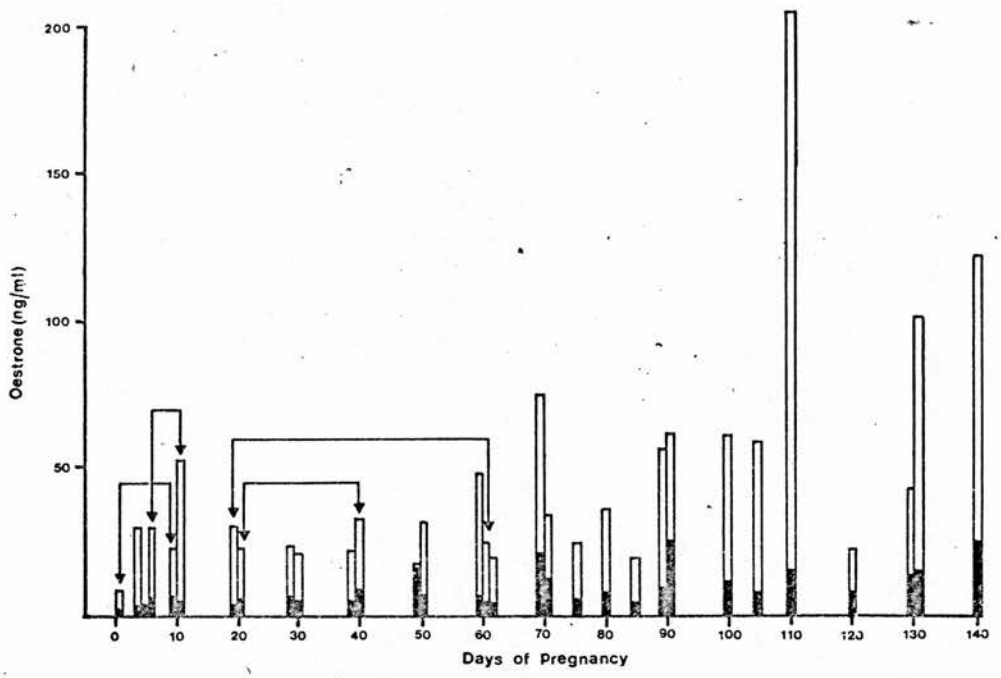


Fig. 5-4: The sum of the oestrone values (ng/ml) in the right and left utero-ovarian vein (▯) and the oestrone value in the maternal peripheral plasma (▮) throughout pregnancy. The arrows link pregnancies from which serial samples were taken.



and trends in the MPV were similar to those reported in Chapter 4.

(c) The relationship to the presence of CL

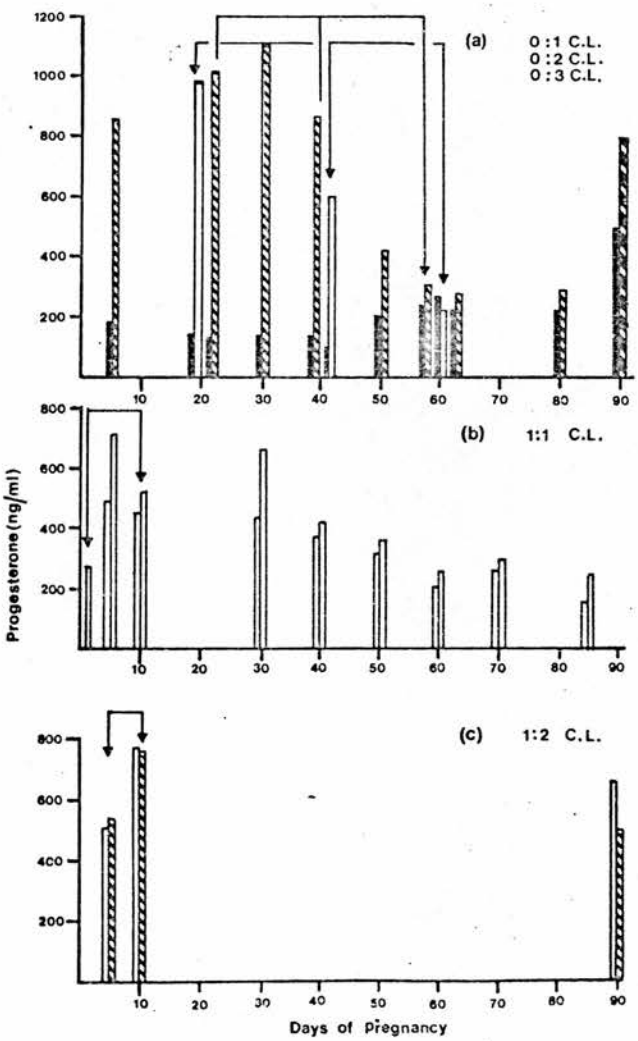
1 to 3 corpora lutea (CL) were found between the 1st and 90th day of gestation. After 90 days it was not generally possible to distinguish the CL (See also Table 4-2). In 21 pregnancies, the CL were distributed as follows: 10 pregnancies had 1 CL on each ovary; 9 had 1-3 CL on one ovary and none on the other; 2 had 2 CL on 1 ovary and 1 on the other. In these 21 pregnancies, 3 had a total of 3 CL, 17 a total of 2 CL and 1 had 1 CL. Since the number of pregnancies with more or less than 2 CL was small, it was not possible to compare the total hormonal value from the 2 UOVs with the total number of CL present.

(i) Progesterone

The progesterone values in the individual UOVs between days 1 and 90 are shown in Figure 5-5. Progesterone values were similar in paired UOV if both drained an ovary containing CL. The pattern for the individual veins was similar to that for the total concentration (section 5.3b-i).

In contrast, progesterone values were nearly always lower (11 out of 12 cases) in the UOV draining an ovary containing no CL than in the contralateral vein draining either 1, 2 or 3 CL. The difference between the 2 veins was significant for the first 50 days of pregnancy ($P<0.001$, paired t-test, $n=7$) but not between 60 and 80 days. The pregnancy at day 90 was excluded because it

Fig. 5-5: Individual progesterone values (ng/ml) in the paired utero-ovarian veins.
(a) 1 vein associated with no corpus luteum (C.L.) (▩) paired with vein associated with 1 (▥), 2 (▧) or 3 (▨) C.L.
(b) both veins associated with 1 C.L. (▥).
(c) 1 vein associated with 1 C.L. (▥) and 1 with 2 C.L. (▧). The arrows link pregnancies from which serial samples were taken.

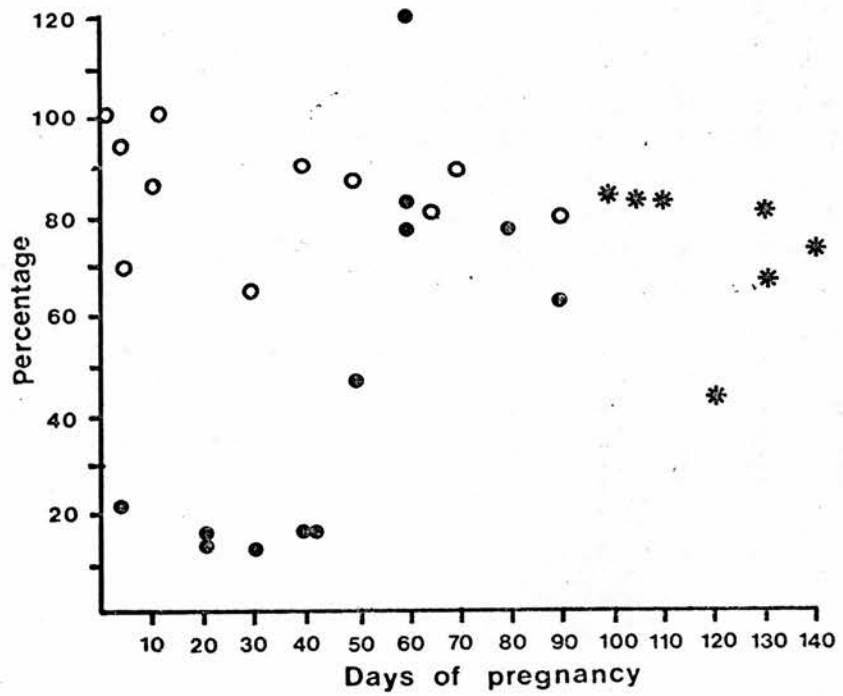


was the only pregnancy in which the contralateral ovary contained 3 CL. Progesterone values in the UOV adjoining the ovary containing no CL were significantly higher than the maternal peripheral levels.

The progesterone trends in the individual UOVs were different depending on the presence or absence of CL in the adjoining ovary. In the vein not associated with corpus luteum, progesterone levels were unchanged until day 40. A gradual increase occurred between 40 and 60 days and at day 60, levels were significantly higher than those in the conception cycle ($P<0.02$, t-test, n=6). In contrast, progesterone values in the vein associated with CL declined approximately 5-fold between days 30 and 60 and at day 60, were significantly lower ($P<0.001$, t-test, n=6) than those in the conception cycle. A different pattern in the UOV between days 20 and 60, depending on the presence or absence of CL, was clearly shown for serial UOV samples taken at days 20, 40 and 60 from 2 individuals.

The relationship between the 2 UOVs is expressed as a percentage in Figure 5-6. In the vein draining the ovary with no CL, progesterone levels were only 10-20% of the contralateral vein until day 40. Between 40-60 days there was an increase in the percentage and after day 60 it was similar to the percentage found in pregnancies where both veins drained an ovary containing a CL.

Fig. 5-6: The progesterone relationship between the 2 utero-ovarian veins expressed as a percentage. Pregnancies with both utero-ovarian veins associated with corpus luteum (C.L.) (lower/higher value) (o). Pregnancies with 1 utero-ovarian vein associated with and 1 not associated with C.L. (●). Pregnancies in which the number of C.L. were not distinguishable (✱).



(ii) Oestradiol

The oestradiol concentrations in the individual UOVs are shown in Figure 5-7. The presence or absence of CL did not affect either the level of oestradiol in the UOV or the pattern of oestradiol in the individual veins with advancing gestation. This was confirmed by samples taken at serial laparotomies.

The relationship between the UOVs, expressed as a percentage, is shown in Figure 5-8. Marked differences between the 2 veins were often observed up to the 60th day of gestation. Whether the levels in the 2 veins were similar or different did not depend on the presence or absence of CL.

(iii) Oestrone

Figure 5-9 shows the oestrone concentrations in the individual UOVs in relation to the number of CL present. Oestrone values were similar in the paired UOVs if both drained an ovary containing a CL. Oestrone values were higher in the UOV associated with CL than in the vein not associated with CL. Like progesterone, the difference was significant up to day 50 of pregnancy ($P<0.01$, t-test, n=6) but not between days 60 and 80. Samples taken at serial laparotomies showed a slight increase between days 20 and 40 and lower 60 day levels than 20 or 40 day levels in both the UOV associated with and not associated with CL.

The relationship between the 2 UOVs is expressed as a percentage in Figure 5-10. Oestrone values in the UOV not associated with a CL were $53.8 \pm 9.7\%$ (s.e.m.,

Fig. 5-7: Individual oestradiol values (ng/ml) in the paired utero-ovarian veins.
(a) 1 vein associated with no corpus luteum (C.L.) (▮) paired with vein associated with 1 (▯), 2 (▩) or 3 (▩) C.L.
(b) both veins associated with 1 C.L. (▯).
(c) 1 vein associated with 1 C.L. (▯) and 1 with 2 C.L. (▩). The arrows link pregnancies from which serial samples were taken.

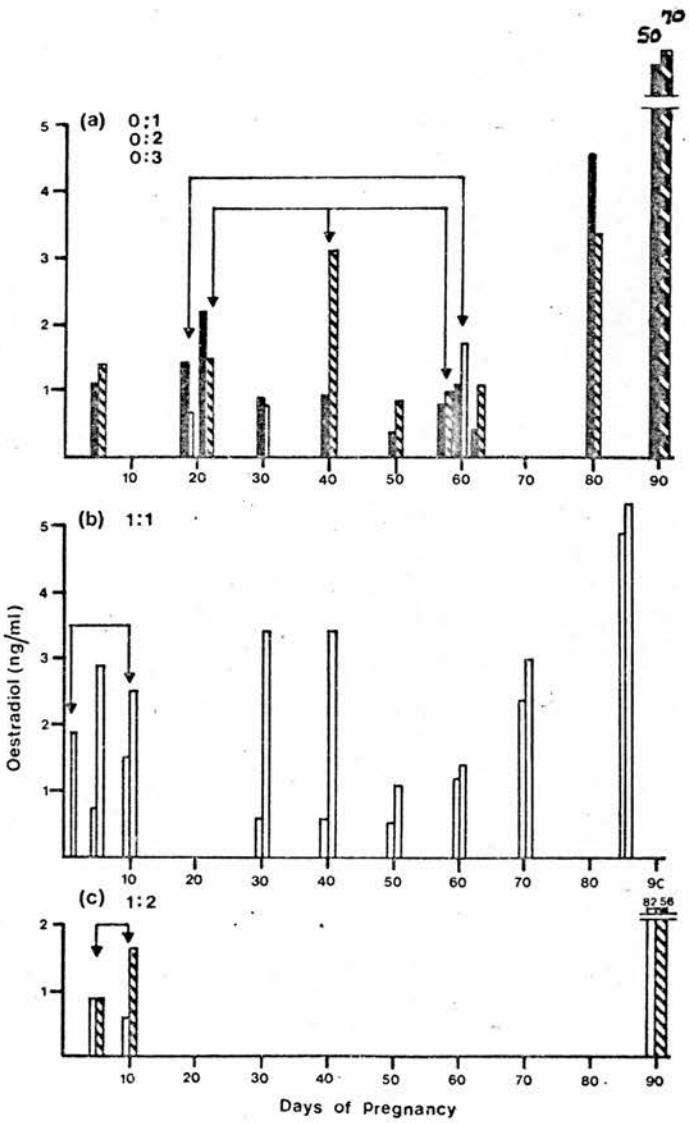


Fig. 5-8: The oestradiol relationship between the 2 utero-ovarian veins expressed as a percentage. Pregnancies with both utero-ovarian veins associated with corpus luteum (C.L.) (lower/higher value) (o). Pregnancies with 1 utero-ovarian vein associated with and 1 not associated with C.L. (●). Pregnancies in which the number of C.L. were not distinguishable (✱).

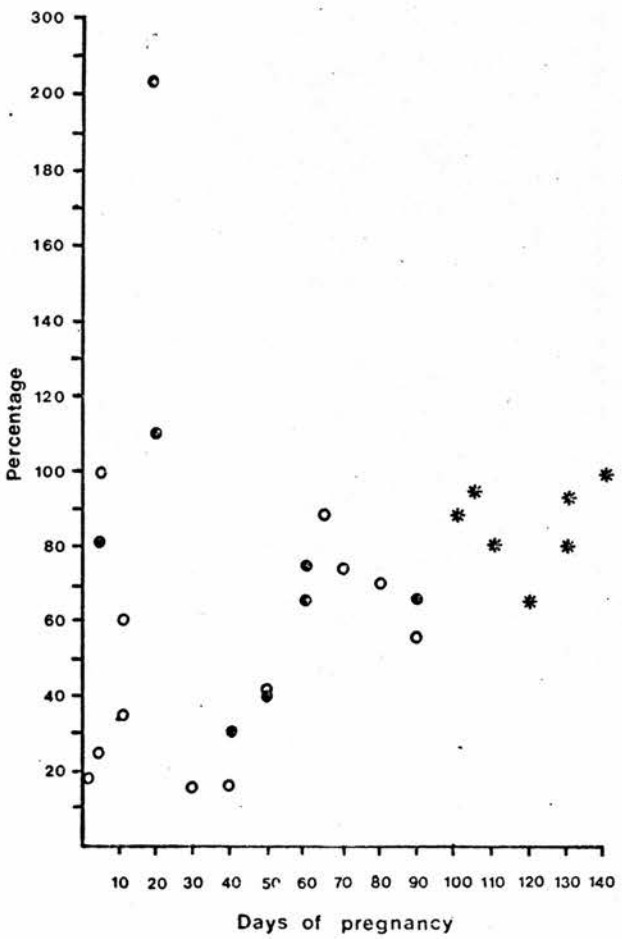


Fig. 5-9: Individual oestrone values (ng/ml) in the paired utero-ovarian veins.
(a) 1 vein associated with no corpus luteum (C.L.) (▮) paired with vein associated with 1 (∩), 2 (▮▮) or 3 (▮▮▮) C.L.
(b) both veins associated with 1 C.L. (∩).
(c) 1 vein associated with 1 C.L. (∩) and 1 with 2 C.L. (▮▮). The arrows link pregnancies from which serial samples were taken.

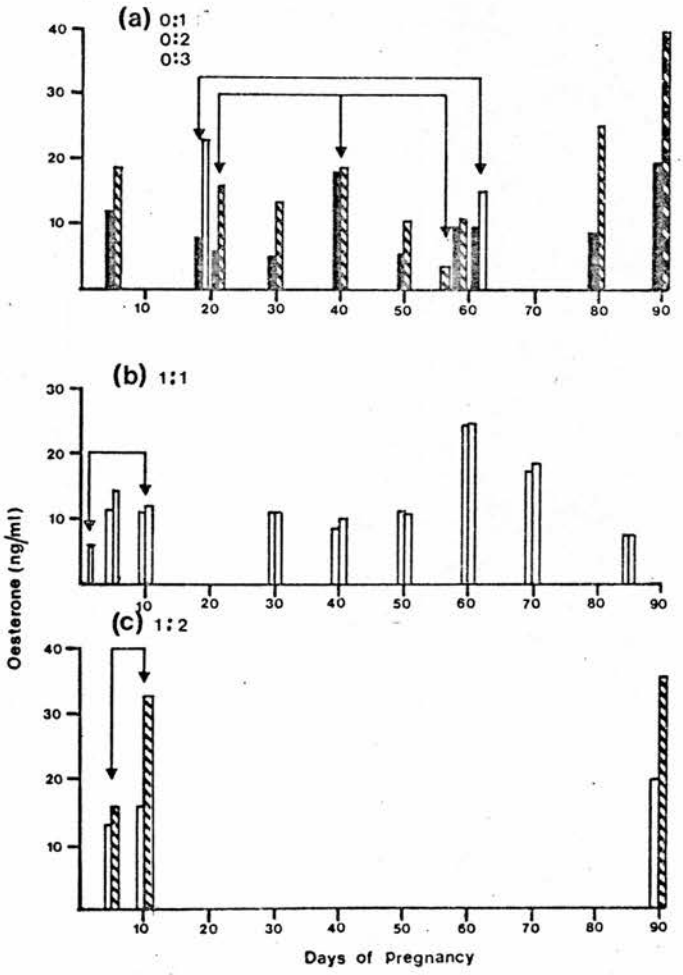
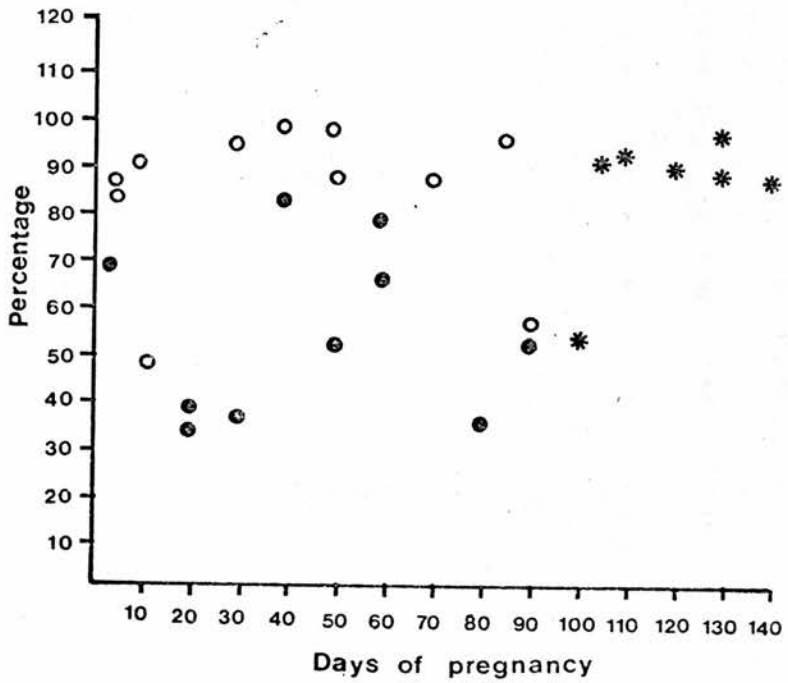


Fig. 5-10: The oestrone relationship between the 2 utero-ovarian veins expressed as a percentage. Pregnancies with both utero-ovarian veins associated with corpus luteum (C.L.) (lower/higher value) (o). Pregnancies with 1 utero-ovarian vein associated with and 1 not associated with C.L. (●). Pregnancies in which the number of C.L. were not distinguishable (✳).



n=9) of the contralateral UOV. When both veins were associated with 1 or more CL, the lower values were 87.0±1.8% (s.e.m., n=13) of the higher. The percentage for the 2 groups tended to converge at 40-60 days of pregnancy although considerable differences between the paired veins persisted in some animals. After 100 days oestrone values in the 2 UOVs were similar.

(d) Relationship between the UOV and MPV

The relationship between the UOV and MPV is expressed as a ratio for each hormone in Figure 5-11a,b and c.

Hormonal levels were always higher in the UOV than in the MPV. Except at 140 days of pregnancy, the ratio was highest for progesterone. The oestradiol and oestrone ratios were generally fairly similar. Whereas, the progesterone ratio declined with advancing gestation, there was no consistent overall trend for the oestradiol and oestrone ratios. The initial decline in the progesterone ratio occurred during the first 60 days of pregnancy. There was a further decline in the ratio after day 120.

Correlations (regression analysis) were calculated between the UOV and MPV concentrations for each hormone. The only correlations ($r>0.7$) were for progesterone (90-140 days, r=0,79, n=8) and oestradiol (0-80 days, r=0.78, n=22; 90-140 days, r=0,76, n=8).

(e) The hormonal ratios

The progesterone to oestradiol ratio is shown in Figure 5-12. Progesterone levels were higher than oestradiol in both the UOV and MPV throughout pregnancy. The ratio declined with advancing gestation but the decline

Fig. 5-11a: The ratio between the progesterone values in the utero-ovarian vein (U.O.V.) and the maternal peripheral plasma (M.P.V.) . The arrows link pregnancies from which serial samples were taken.

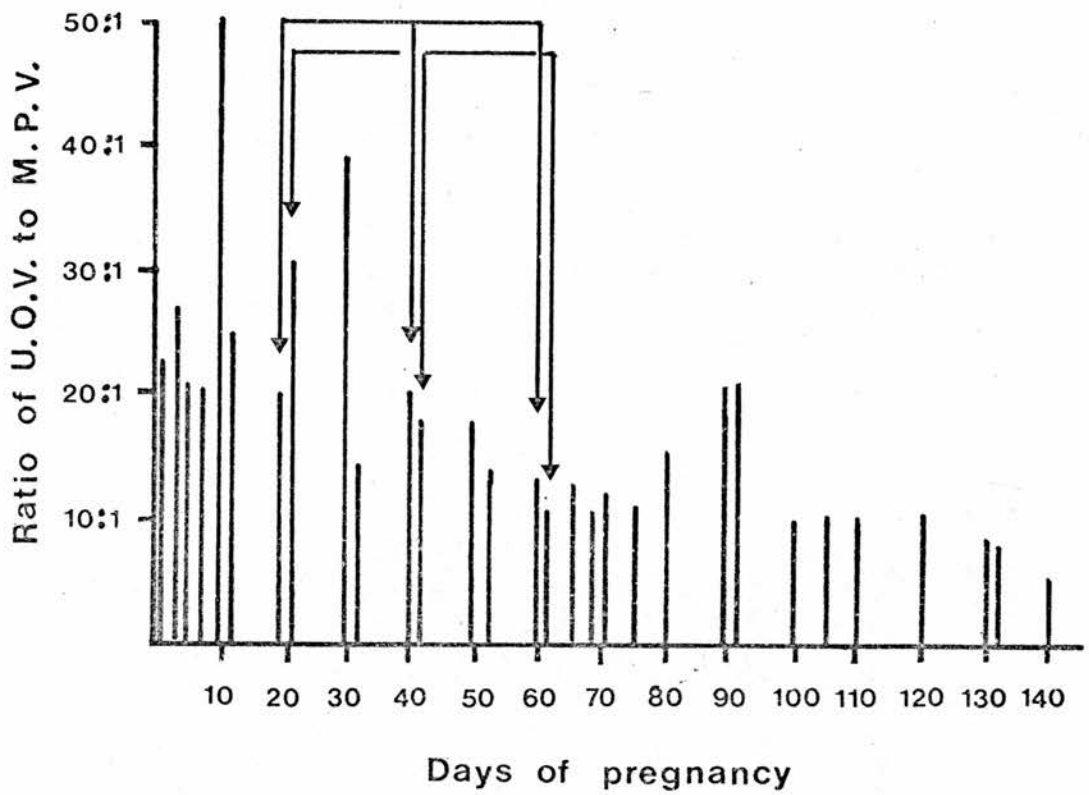


Fig. 5-11b: The ratio between the oestradiol values in the utero-ovarian vein (U.O.V.) and the maternal peripheral plasma (M.P.V.)

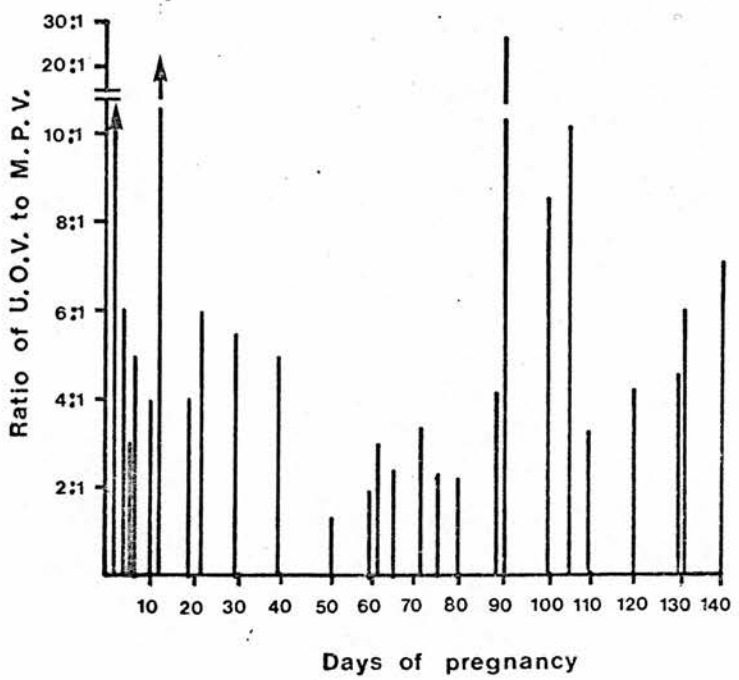


Fig. 5-11c: The ratio between the oestrone values in the utero-ovarian vein (U.O,V.) and the maternal peripheral plasma (M.P.V.)

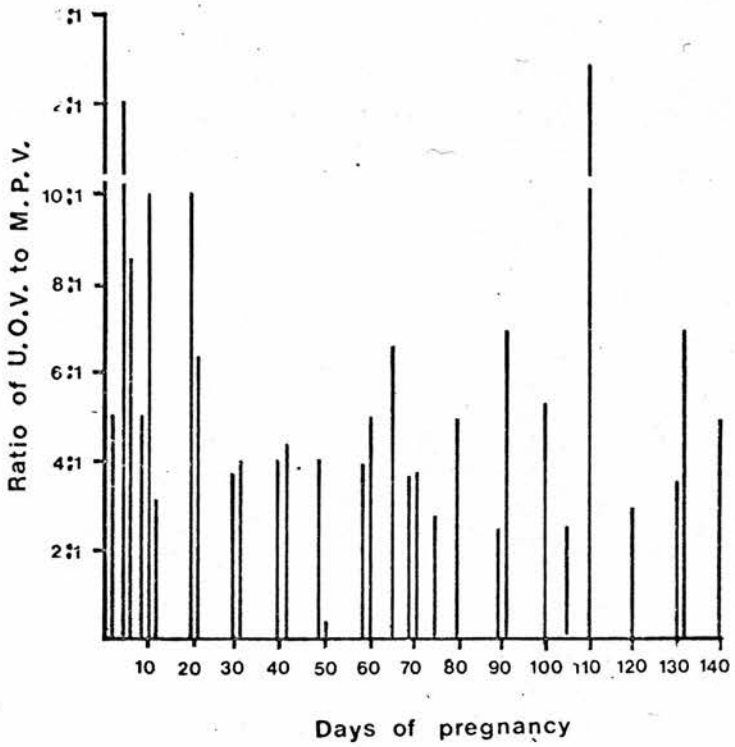


Fig. 5-12: The ratio of progesterone to oestradiol in the utero-ovarian vein (●) and in the maternal peripheral vein (o).

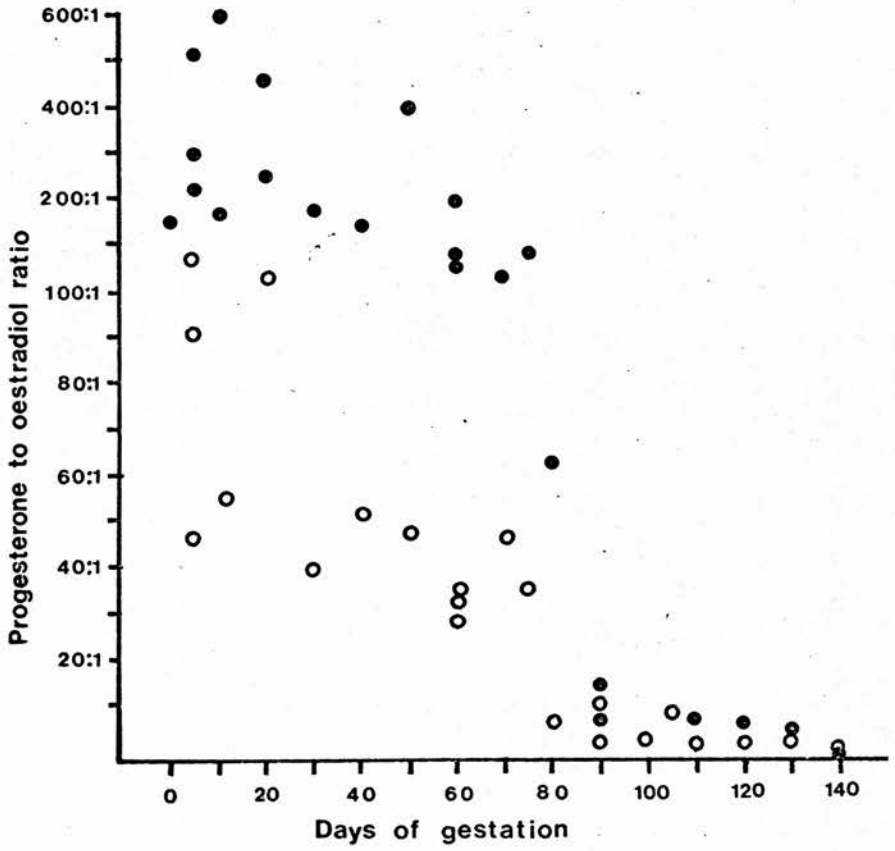


Fig. 5-13: The ratio of oestradiol to oestrone in the utero-ovarian vein (●) and in the maternal peripheral vein (o). The data from the 80 day pregnancy is repeated for reference.

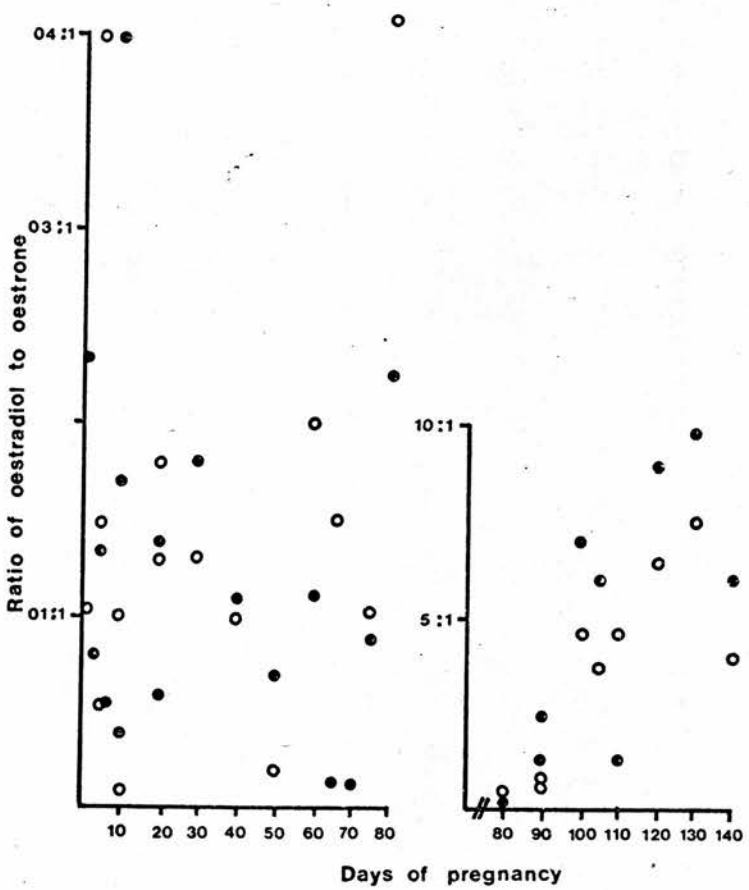
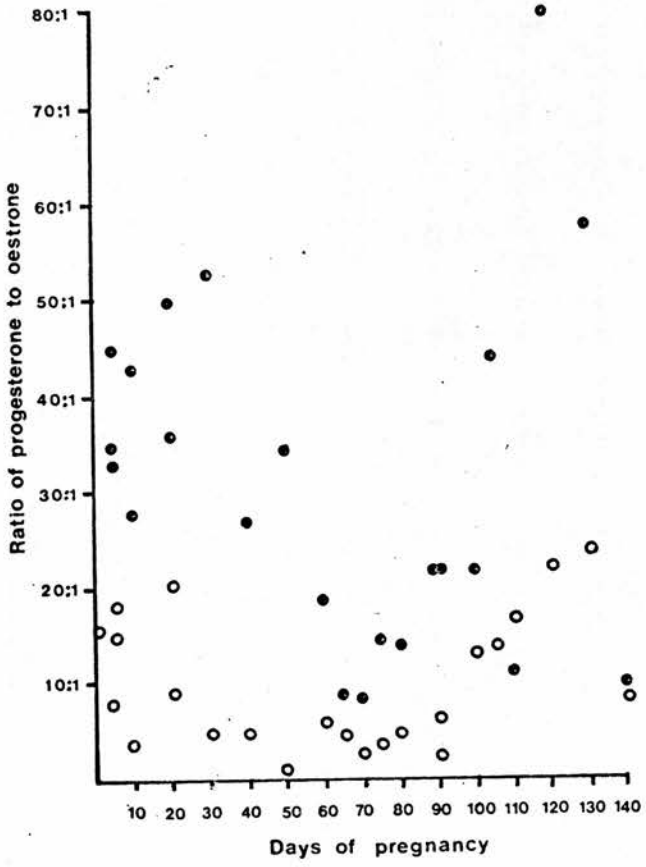


Fig. 5-14: The ratio of progesterone to oestrone in the utero-ovarian vein (●) and in the maternal peripheral vein (o).



was steeper in the UOV. The ratio was generally higher in the UOV than in the MPV.

The oestradiol to oestrone ratio is shown in Figure 5-13. Oestrone was the predominant oestrogen in the UOV up to day 80 of pregnancy. There was no consistent trend in the UOV ratio prior to day 80 and no consistent relationship to the MPV ratio. After day 80 there was a sustained increase in the ratio and oestradiol was the major oestrogen.

The progesterone to oestrone ratio is shown in Figure 5-14. The ratio in the UOV declined between approximately 30-80 days of pregnancy and then gradually increased during the latter half of gestation. The ratio in the UOV was generally higher than that in the MPV.

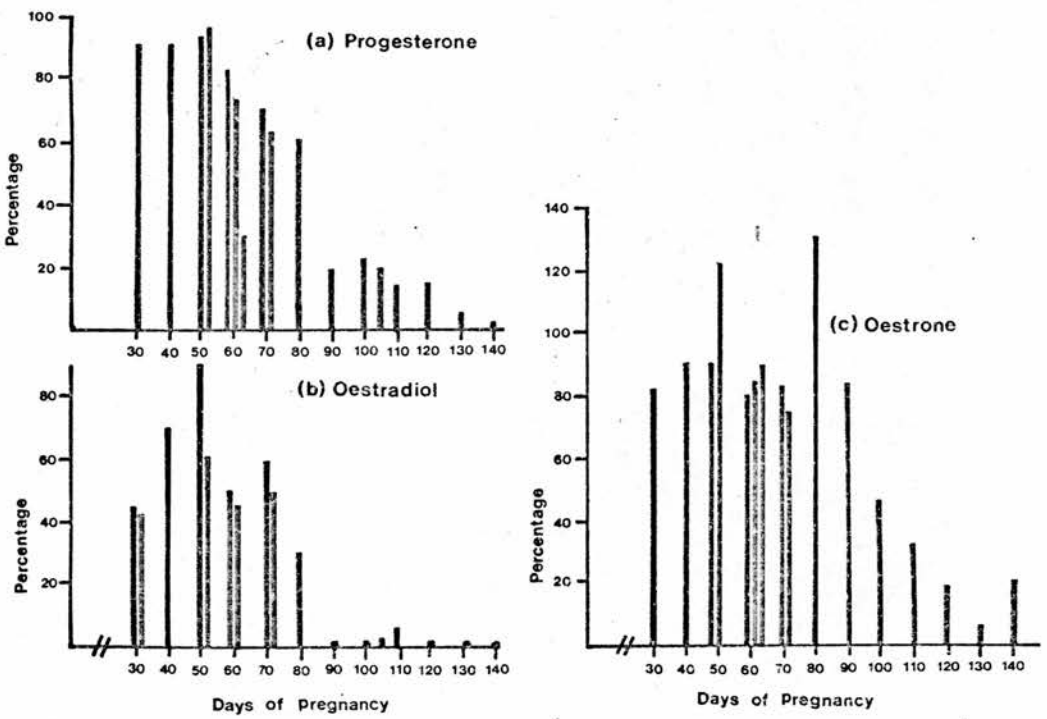
There was no correlation between progesterone and oestradiol, between oestradiol and oestrone or between progesterone and oestrone before or after 90 days of pregnancy.

(f) The effect of removing the feto-placental unit

The hormonal values in the paired UOVs before and after removal of the feto-placental unit (f.p.) is expressed as a percentage (post/pre f.p.%) in Figure 5-15. Only pregnancies from which samples were taken from both UOVs before and after unit removal are included.

For progesterone, the percentage was $>90\%$ up to day 50. There was a gradual decline in the percentage between 50-80 days to around 60%, with 1 pregnancy at day 60 having a lower percentage of 30%. A steep decline

Fig. 5-15: The percentage of progesterone (a), oestradiol (b), and oestrone (c) remaining in the utero-ovarian vein following removal of the feto-placental unit. 100% equals the pre-removal value.



between days 80-90 resulted in the percentage dropping to < 20%. The decline continued until the end of pregnancy.

The oestradiol percentage was variable for the first 80 days of pregnancy (range 30-90%). There was a sharp decline in the percentage at 80-90 days and the percentage was < 6% for the remainder of pregnancy.

The oestrone percentage remained high (75-130%) for the first 90 days. After day 90, the percentage declined.

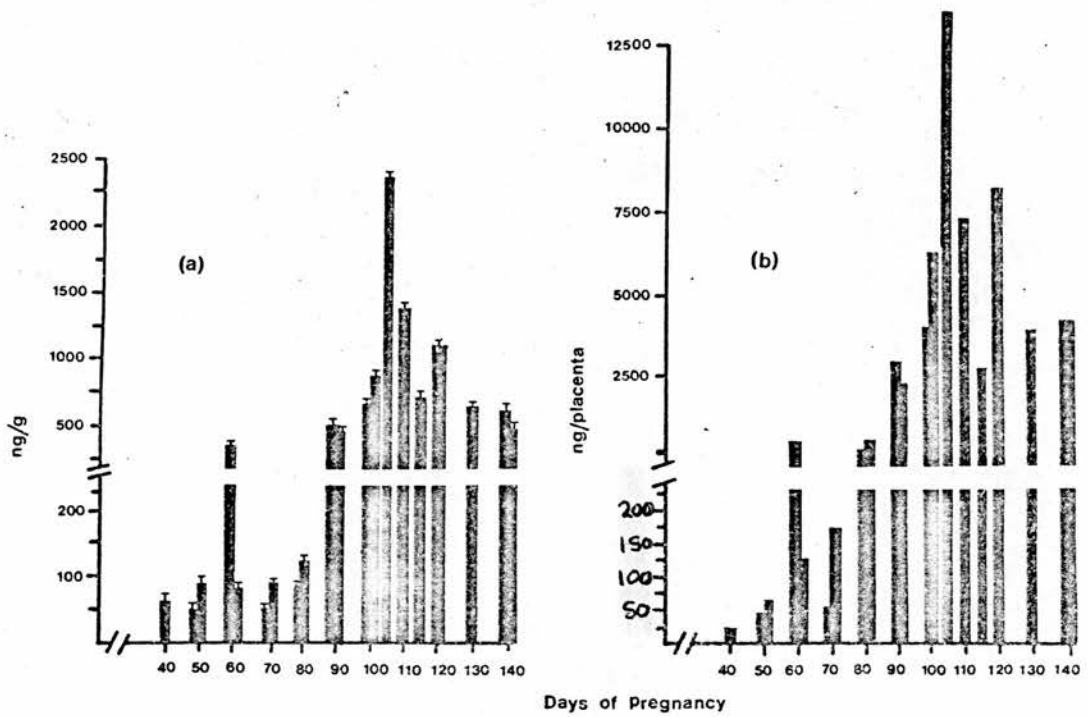
The actual hormonal value in the UOVs following removal of the feto-placental unit were similar to those in the MPV following unit removal after day 90 for progesterone and oestradiol and after day 100 for oestrone. These UOV values were also similar to the UOV values not associated with CL in early pregnancy for progesterone and oestrone.

Part II: The hormonal concentration and content in placental tissue

(a) Progesterone

The placental progesterone concentration and content is given in Figure 5-16. With the exception of 1 pregnancy at day 60, there was little change in concentration between 40-80 days. The progesterone concentration in the 60 day pregnancy was about 5 times the mean value for the other 40-80 day pregnancies. This was the same animal which showed a relatively low post/pre f.p.% (Figure 5-15).

Fig. 5-16: The progesterone concentration (±s.e.m.)(a) (ng/g), and the progesterone content in the placenta (ng/placenta)(b)



In contrast to the concentration, the content increased approximately 10-fold between days 40-80. The pregnancies with the higher values showed the lower post/pre f.p.%

The concentration and content increased about 4 to 5-fold respectively at 80 to 90 days. Maximum values were found between 105 and 120 days. The low content value at 115 days was from a singleton pregnancy, in which the placental weight was less than for multiple pregnancies (Chapter 7). The concentration and content at 130 to 140 days was similar to day 90 values. Values were significantly higher after day 90 than before day 90 ($P<0.001$, t-test, n=19).

(b) Oestradiol

The placental oestradiol concentration and content is given in Figure 5-17. The concentration increased about 3 to 8-fold and the content about 25 to 85-fold between 40 to 80 days. However, there was considerable interanimal variation in values. Between 80 to 90 days, there was approximately a 18 to 21-fold increase, which was about 4 times the progesterone increase which occurred at this time. The highest concentrations and contents were found at 140 days, when values were 3-5 and 3-8 times respectively the mean 90-130 day values. Values were significantly higher after day 90 than before day 90 ($P<0.001$, t-test, n=18).

(c) Oestrone

The oestrone concentration and content is shown in Figure 5-18. There was approximately a 3 and 30-fold increase in concentration and content respectively between 40 and 80 days. Like oestradiol, there was considerable

Fig. 5-17: The oestradiol concentration (±s.e.m.) (a) (ng/g), and the oestradiol content in the total placenta (ng/placenta). (b)

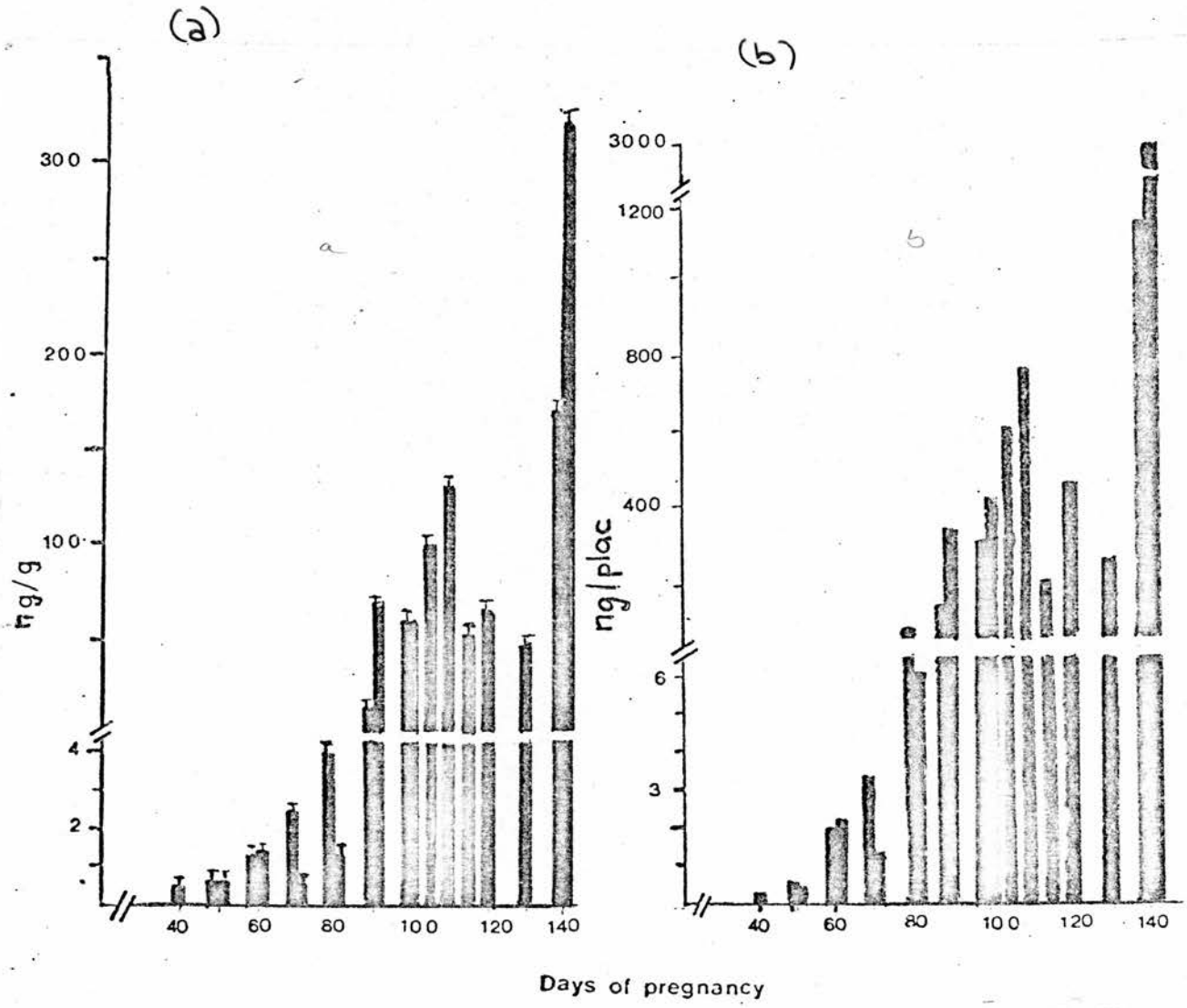
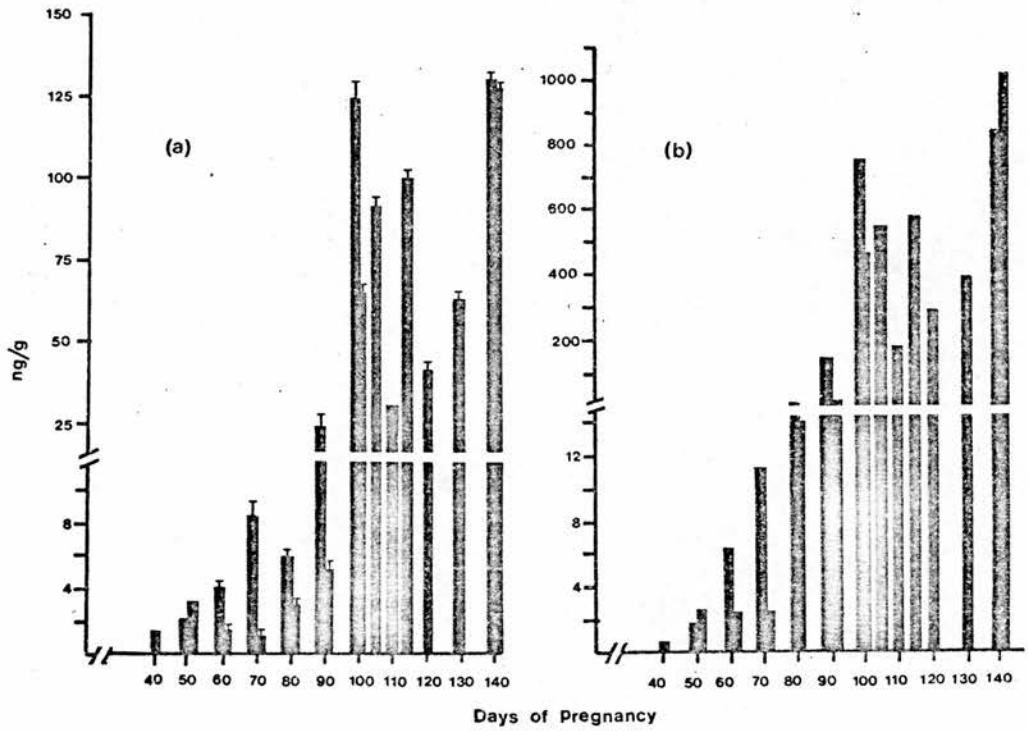


Fig. 5-18: The oestrone concentration (±s.e.m.) (ng/g), (a) and the oestrone content in the total placenta (ng/placenta) (b)



interanimal variation in the oestrone values. There was a steep increase in the concentration and content between 80-100 days of pregnancy and thereafter values remained high until the end of pregnancy. Values were significantly higher after day 90 than before day 90 ($P<0.01$, t-test, n=18).

(d) Androstenedione

Figure 5-19 shows the androstenedione concentration and content in placental tissue. The concentration before day 90 was significantly lower ($P<0.05$, t-test, n=20) than that found after day 90 although there was some overlap between the 2 groups. Although there was a definite increase at 90 days, within each period (days 40-80 and 90-140) there was no consistent change in values with advancing gestation.

The content increased gradually between 40-80 days followed by a sharp increase at 80-90 days. Thereafter levels followed a similar pattern to the concentration and were significantly higher ($P<0.001$, t-test, n=20) than the levels before day 90.

(e) Testosterone

Figure 5-20 shows the testosterone concentration and content in placental tissue. There was a gradual increase in both the concentration and content until approximately day 100. The concentration and total amount was significantly higher after day 90 than between 40 and 80 days ($P<0.01$, t-test, n=18).

(f) LH/CG

The LH/CG concentration and content is given in Figure 5-21. The concentration declined 4000-fold

Fig. 5-19: The androstenedione concentration (±s.e.m.)(a) (ng/g) and the androstenedione content in the total placenta (ng/placenta)(b)

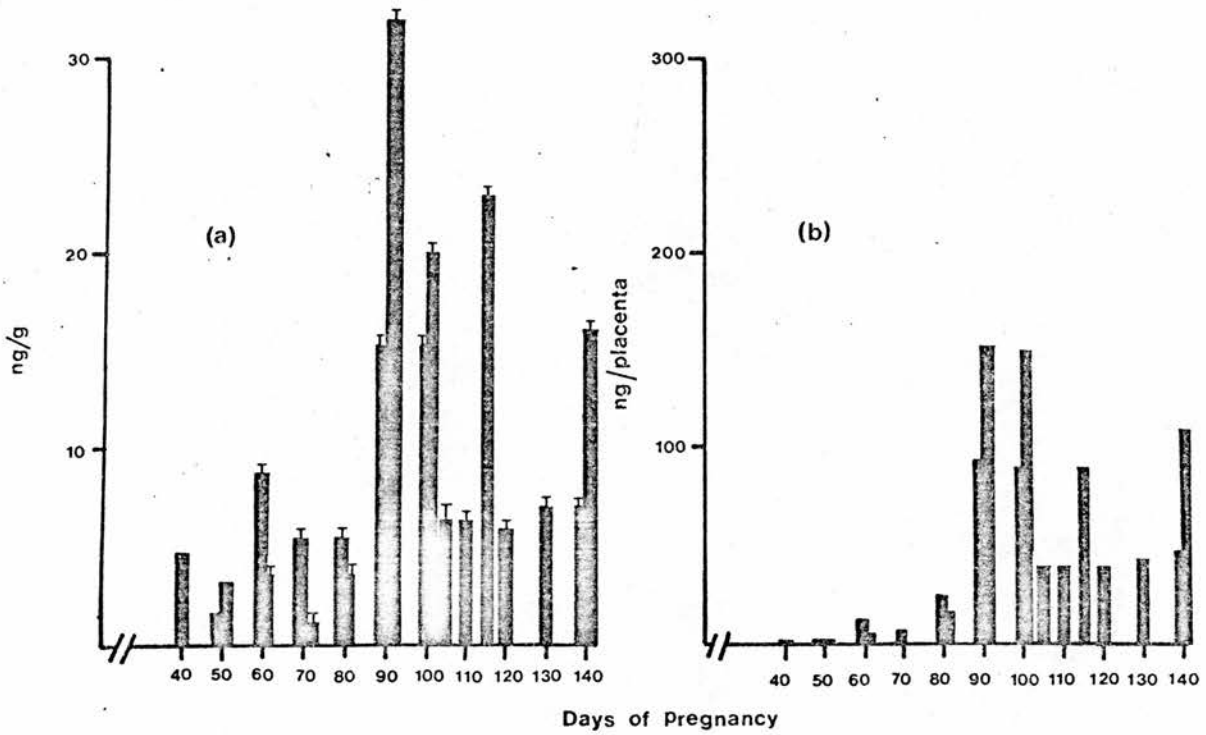


Fig. 5-20: The testosterone concentration (±s.e.m.)(a) ng/g) and the testosterone content in the total placenta (ng/placenta).(b)

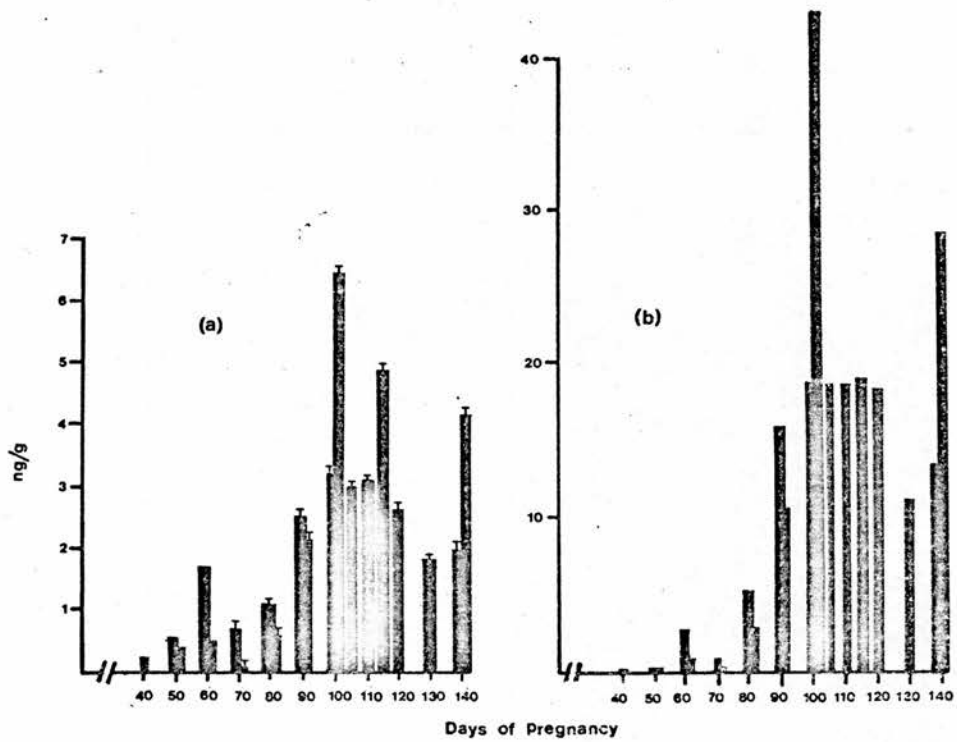
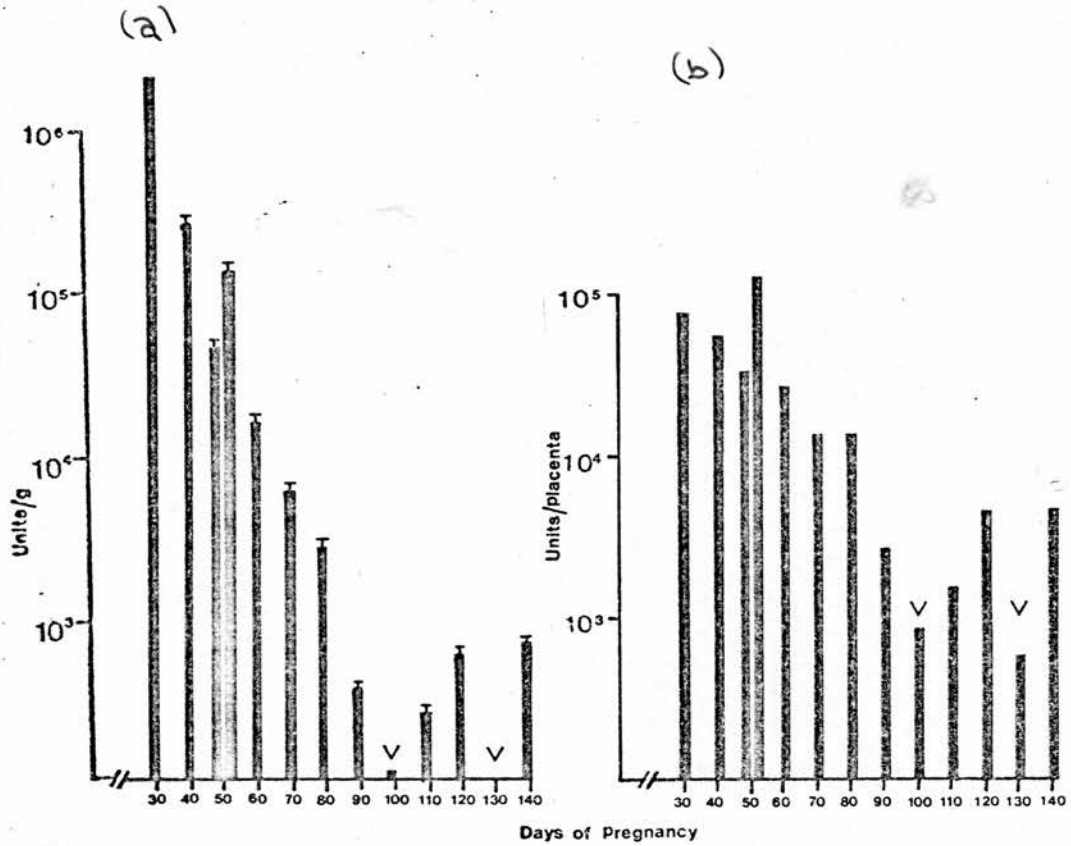


Fig. 5-21: The LH/CG concentration (±s.e.m.) (ng/g)(a) and the LH/CG content in the total placenta (ng/placenta)(b)



between 30 and 90 days. Concentrations remained low after day 90 and were undetectable in a few instances. The content of LH/CG did not decline until after day 70 when maximum values were found. Thereafter, the LH/CG content followed the same trend as the concentration although the decline was less steep.

(g) Comparison of the placental discs

The hormonal concentration and content between the 2 placental discs were compared after day 60 (t-test). Prior to day 60, there was too little tissue available from each disc for statistical comparison. Since the results were similar for concentration and content, the data hereafter will refer only to concentrations. Table 5-1 shows the pregnancies in which there was a significant difference between the discs for any of the hormones. The majority of pregnancies did not show a significant difference and there was no consistent association of hormones. In order to confirm these results, new homogenates were prepared and extracted from 8 pregnancies; 4 with a signficant difference and 4 without a significant difference. The results were confirmed in all cases.

The fetal attachments were analysed and are given in Table 5-2 for pregnancies in which there was a significant difference. No consistent relationship is evident. Although females were associated with the disc with higher progesterone and oestradiol concentration, the presence of a female(s) did not necessarily result in higher values (e.g. day 140 and 115). The disc with the higher testosterone value may also be associated with a

Table 5-1 The stage of gestation and the animals in which a significant difference in at least 1 hormone concentration was found. The + and - sign refer to a significantly higher or lower concentration for the same placental disc. The blanks signify no significant difference. The number of animals tests (a), the number (b) and percentage (c) of animals with a difference is also given.

Day of gestation	No. of hormones	Progesterone	Oestradiol	Oestrone	Androstenedione	Testosterone
70	4		+	-	+	+
80	2				+	+
100	3	-			+	+
100	3	+	+			+
115	2	-				+
120	4	-	-	+		+
140	1		+			
(a) No. tested		16	16	16	13	13
(b) No. with diff.		4	4	2	3	6
(c) % with diff.		25	25	12	23	48

Table 5-2 The number and sex of the fetuses attached to the placental discs in which a significant difference in hormone concentration was found. The fetal attachments given on the left hand side are for the disc with the higher hormone value (+) and that on the right hand side for the disc with the lower hormone value (-). M=male fetus; F=female fetus. Subscripts denote different fetuses of the same sex. Abbreviations as for Table 5-3.

		P4		OE2		OE1		A		T	
Day of gest.	Anim. No.	+	-	+	-	+	-	+	-	+	-
70	48W			F	M	M	F	F	M	F	M
80	46W							M	F	M	F
100	44W	M_1F_1	M_1F_1					M_1F_1	M_1F_1	M_1F_1	M_1F_1
100	6R	F_1F_2	F_1	F_1F_2	F_1					F_1F_2	F_1
115	11W	F	none							none	F
120	10W	F_2	M_1F_1	F_2	M_1F_1	M_1F_1	F_1			M_1F_1	F_2
140	60W			F_1F_2	M						

male, or female fetus, both or none at all.

(h) Comparison with maternal values

The overall profile of progesterone and oestradiol in the placental tissue resembled that in the peripheral plasma with a steep increase at 90 days and a decline (progesterone) or increase (oestradiol) at 140 days (see Chapter 4). The increase in oestrone placental values was not necessarily reflected in peripheral values. The androstenedione and testosterone profiles in the placental tissue differed from the MPV. Maximum values in the placenta occurred during the latter half of pregnancy whereas they occurred at 6-10 weeks in the MPV.

The ratio between the hormonal concentration in the placenta and the MPV is shown in Table 5-3. Hormonal levels were generally higher in the placental tissue than in the MPV for all hormones after 90 days. The mean ratio after 90 days was significantly higher ($P<0.001-0.05$, t-test) than before 90 days for all hormones. The ratio varied between hormones and was highest for testosterone after 90 days.

There was no correlation between the placental concentration and MPV for any of the hormones either before or after 90 days. The placental content was correlated with MPV levels for progesterone and oestradiol after 90 days. ($r = 0.75$, $P<0.01$, $n = 11$; $r = 0.80$, $P<0.01$, $n = 11$ respectively.)

Table 5-3 The ratio between the placental concentration and the maternal peripheral plasma for progesterone (P4), oestradiol (OE2), oestrone (OE1), androstenedione (A) and testosterone (T).

Day	P4	OE2	OE1	A	T
40	0.97		1.65	0.67	0.12
50	1.18	0.70	0.04	0.17	0.88
50	1.14	1.29	1.88	0.26	0.37
60	10.3	1.30	1.60	0.7	1.85
60	0.97	2.78	0.78	0.24	0.87
70	0.65	1.77	0.43	0.6	0.53
70	1.56	0.45	0.82	0.14	0.17
80	1.18	3.15	0.79	0.77	5.54
80	3.91	0.33	0.40	0.55	2.05
85	2.01	0.85	0.21	-	-
90	8.89	2.72	-	1.87	15.91
90	7.09	3.50	0.18	5.33	11.72
100	5.67	1.21	7.18	1.67	26.93
100	5.78	1.10	5.33	2.00	40.00
105	9.48	3.74	1.57	0.72	18.10
110	4.81	1.71	2.13	0.96	1.07
115	4.21	1.82	6.25	2.87	24.00
120	6.89	1.42	6.09	0.63	18.60
130	2.28	0.50	5.25	0.89	12.55
140	3.05	1.71	5.30	1.01	17.00
140	5.04	2.56	11.90	2.7	41.14
Mean ±s.e. (40-85)	2.38±0.91	1.40±0.30	0.86±0.21	0.46±0.08	1.37±0.51
Mean ±s.e. (90-140)	5.75±0.66	1.99±0.23	5.11±1.00	1.87±0.42	20.6±3.6

(i) Comparison with the utero-ovarian and umbilical vein

Figure 5-22 shows ratio between the placental and UOV hormone concentrations. Whereas progesterone and oestradiol were lower in the placental tissue than in the UOV, oestrone was generally higher after 100 days of pregnancy. The progesterone and oestrone ratio was significantly higher after 90 days than before 90 days ($P<0.02$, t-test). There was no consistent change in the oestradiol ratio.

Placental hormonal concentrations or content were not correlated with UOV values before or after 90 days for any hormones.

Umbilical vein (UV) samples were available only after 90 days (Chapter 6). The ratio between the hormonal concentration in the placenta and the UV is shown in Figure 5-23. The ratio declined with advancing gestation for all 3 hormones. The ratio was generally highest for oestradiol and except at 140 days, lowest for progesterone. The ratio of the placental concentration to the UV was greater than the ratio to the UOV for progesterone and oestradiol. The reverse was found for oestrone.

The placental content or concentration were not correlated with UV values for any of the hormones.

(j) The hormonal ratios

The ratios between the hormones in the placental tissue and MPV is shown in Table 5-4. Progesterone levels

Fig. 5-22: The ratio between the placental (ng/g) and the utero-ovarian vein (ng/ml) values for progesterone, oestradiol and oestrone.

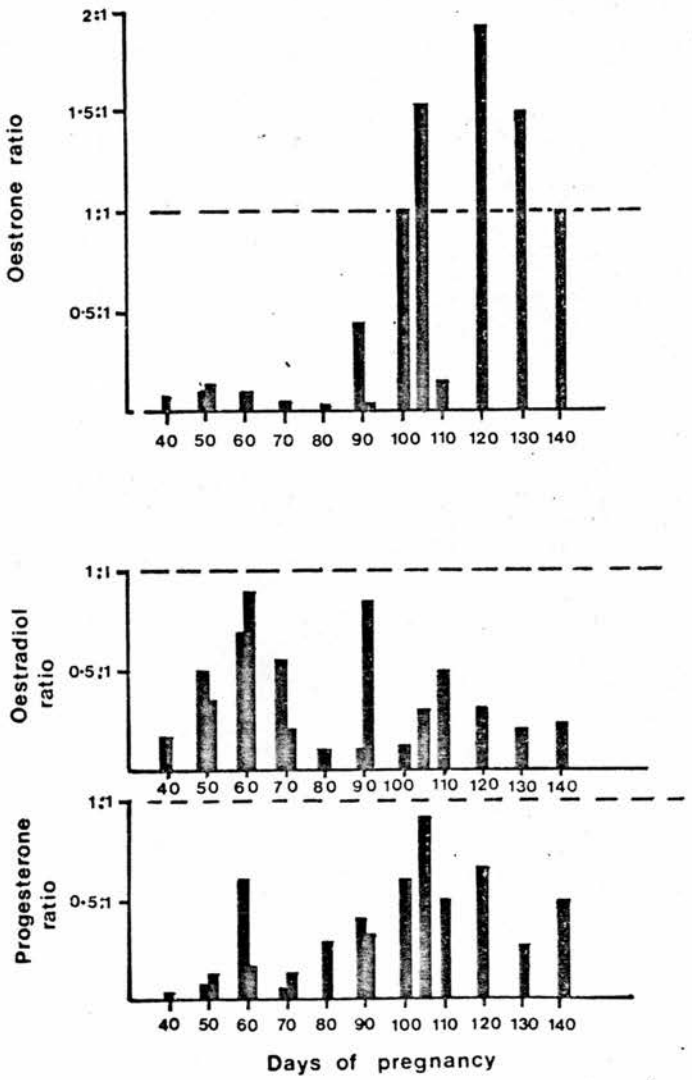


Fig. 5-23: The ratio between the placental (ng/g) and the umbilical vein (ng/ml) values for progesterone, oestradiol and oestrone.

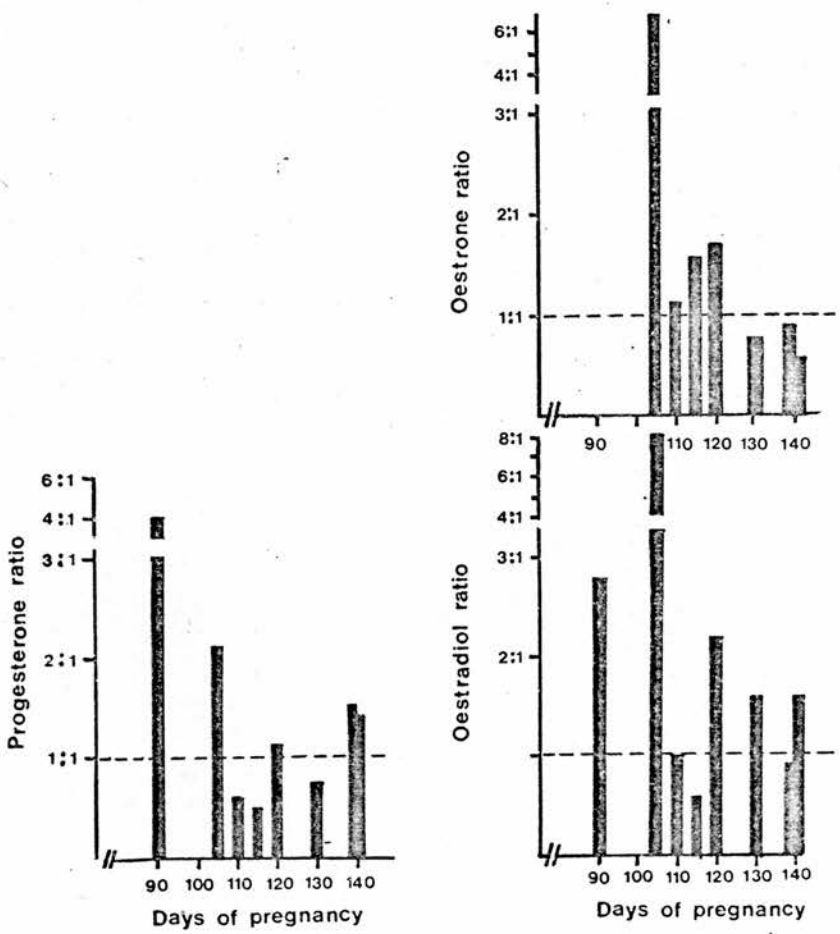


Table 5-4 The hormonal ratios in the placental tissue (plac) and maternal plasma (MPV) for progesterone to oestradiol (P4:OE2); oestradiol to oestrone (OE2:OE1) and androstenedione to testosterone (A:T).

Day of gest.	P4:OE2		OE2:OE1		A:T	
	Plac	MPV	Plac	MPV	Plac	MPV
40	126		0.32		18.76	8.75
50	79	47	0.32	0.02	3.32	16.7
50	138		0.19		7.97	15.0
60	263	33	0.33	0.33	4.87	8.0
60	58	166	0.90		6.34	15.0
70	18	47	0.30	0.07	7.50	5.00
70	130	38	0.52	0.94	6.87	4.00
80	21	56	0.65	0.16	4.70	7.
80	101	9	0.42	0.52	5.62	4.28
90	34	11	0.58	0.33	5.88	8
90	6	3	15.56	0.80	14.67	6.67
100	13	3	0.40	2.39	4.73	12.86
100	15	3	0.87	4.25	3.13	8.33
105	23	9	1.11	0.46	2.16	9
110	10	3	3.97	4.93	2.15	7.78
115	14	6	0.51	1.70	4.75	10.00
120	17	3	1.52	6.42	2.15	9.00
130	14.	3	0.72	7.54	3.92	8.89
140	4	2	1.35	4.26	3.52	8.75
140	1.4	0.7	2.84	13.20	3.80	8.58

exceeded oestradiol levels with only 1 exception (MPV: day 140). The progesterone ratio in the placenta was generally higher, but showed similar changes, as that for the peripheral plasma (Chapter 4) with an overall decline with advancing gestation. Unlike the MPV, placental oestradiol levels did not necessarily exceed oestrone levels after 90 days. The placental oestradiol to oestrone and androstenedione to testosterone ratios were generally lower than those in peripheral plasma after day 90.

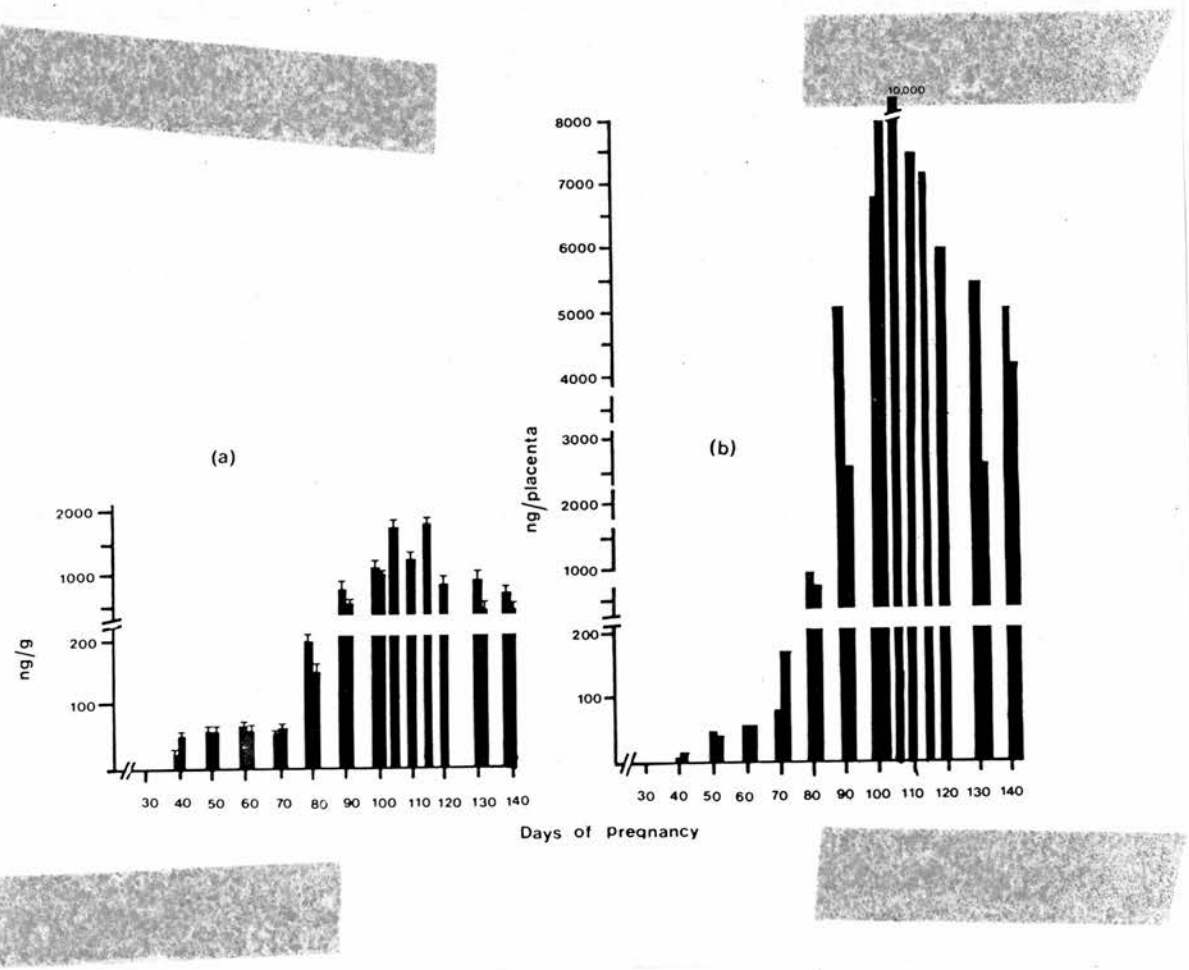
Like the peripheral plasma, progesterone and oestradiol levels in the placenta were correlated between 90-130 days ($r = 0.75$, $P<0.02$, $n = 9$) and androstenedione and testosterone were correlated after 90 days ($r = 0.74$, $P<0.02$, $n = 20$). Oestradiol and oestrone were not correlated.

Part III: Placental secretion of progesterone and LH/CG in vitro

The secretion is expressed as concentration (ng/g or units/g of placenta tissue) or as total amount (concentration times placental weight).

The progesterone secretion during the 1st 24 hours of culture is shown in Figure 5-24. Progesterone was not measurable in the 30 day culture. The concentration remained relatively constant until day 70 followed by a gradual increase at day 80, a steep increase at day 90 and a decline at the end of pregnancy. The total amount

Fig. 5-24: The secretion of progesterone per gram of tissue (a) and per placenta (b) on the first day of culture. (± s.e.m.)



secreted showed a similar pattern except that there was a gradual increase during the first 70 days followed by a steeper increase at 70-100 days. The placental secretion at day 140 was similar to that at day 90.

The secretion of LH/CG during the first 24 hours of culture is shown in Figure 5-25. There was a decline in the concentration between 30 and 80 days. The total amount secreted remained relatively constant until 70 days followed by a decline. LH/CG was not measurable after day 80.

Progesterone and LH/CG were measured for up to 5 days of culture. An exmple of the secretion pattern is shown for 2 placentae in Table 5-5a and the mean percentage of hormone secreted relative to the previous day is shown for all placentae in Table 5-5b. There was a decline in the progesterone secretion with successive days of culture. The steepest decline, occurred between the 1st and 2nd day. LH/CG levels also declined during the first 3 days in culture and then tended to stabilise and increase during the 4th to 5th day. The changes in progesterone and LH/CG secretion with advancing gestation were similar on days 2 to 5 of culture as for Day 1 (Figure 5-24,25) except that the actual levels were lower and that progesterone was not measurable on days 3 to 5 of culture from 40 day placentae.

The pattern of progesterone secretion during the 1st and 3rd day of culture for 2 stages of gestation is shown in Figure 5-26. Two sets of cultures were used; one set for the first 12 hour period and another for the 12 to

Fig. 5-2 5. The secretion of LH/CG per gram of tissue (±s.e.m.) (a) and per placenta (b) on the first day of culture.

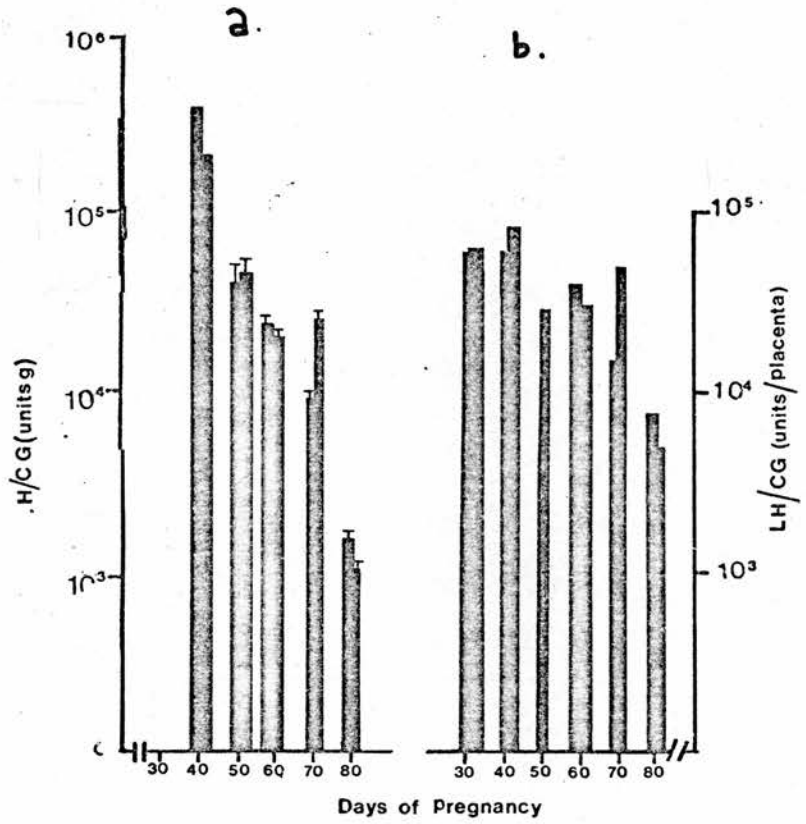


Fig. 5-26: The secretion of progesterone per gram of tissue at 4 hourly intervals during the first day of culture (a and b) and the third day of culture (c).

— 120 days
--- 140 days

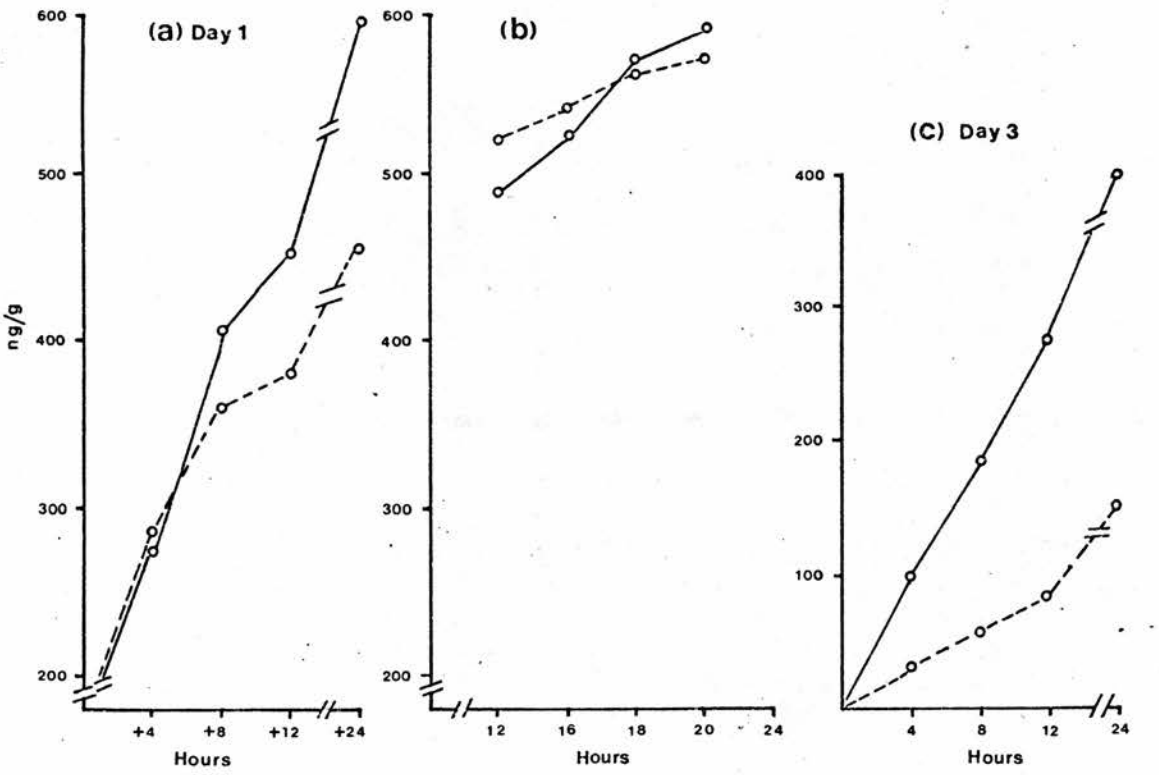


Table 5-5(a) The progesterone (P4) (ng/g ± s.d.) and LH/CG (units/g ± s.d.) secreted during the 1st 5 days of culture for 2 stages of gestation (day 40 and day 80).

	Days of culture				
80 day	1	2	3	4	5
LH/CG	1100±5	825±150	528±40	800±76	1342±100
P4	141±11	65±6	47±5	124±2	17±1
40 day					
LH/CG	$4x10^5$± $5x10^4$	$3.2x10^5$± $9.8x10^4$	$2.1x10^5$± $5.3x10^3$	$2.2x10^5$± $2.5x10^4$	$2.0x10^5$± $1.5x10^4$
P4	50±5	9±1	und	und	und

Table 5-5(b) The mean percentage (±s.d.) for progesterone (P4) and LH/CG on successive days of culture compared to the previous day. P4: n≥12; LH/CG: n≥9.

	Days of culture				
	1	2	3	4	5
P4	100%	31±4	45±6	57±5	68±4
LH/CG	100%	77±20	65±5	81±7	116±30

24 hour period. This was to minimise disturbance to the system and cumulative errors. Previously an hourly sampling regime had been used but the coefficient of variation within each set of cultures was very high. There was an accumulation of progesterone in the medium with time. During the first day, the steepest increase occurred during the first 4 hours of culture. The increase was more steady by the third day of culture.

To test if removal of the medium would affect progesterone secretion, either by removal of progesterone or other products in the medium, or by the renewal of the medium, the culture medium was changed every 4 hours. The results in Table 5-6 show that the amount of progesterone secreted during 1 day of culture is similar for the 2 groups.

To see if increasing the amount of progesterone in the medium has an effect on progesterone or LH/CG secretion, exogenous progesterone was added to 70, 80 and 140 day cultures. The results for 70 and 80 days are given in Table 5-7. There was no effect on progesterone secretion. 4 hourly samples taken during the 1st and 3rd day of culture at 140 days also showed no difference in progesterone secretion. LH/CG was significantly depressed during the first 4 days of culture for the 70 day placenta. No effect on LH/CG secretion was found at 80 days of pregnancy although the amount of progesterone added was similar. LH/CG was unmeasurable in the 140 day cultures.

The long and short term effect on progesterone secretion of pregnenolone in shown in Table 5-8. The

Table 5-6 Comparison of the amount of progesterone (ng/g ± s.d., n = 5) secreted with the culture media completely renewed every 4 hours and the undisturbed controls (n = 5). Placental tissue was from a pregnancy at 110 gestation.

	Hours of culture						
	0-4	4-8	8-12	12-16	16-20	20-24	Total
Media changed	474±41	357±26	192±25	143±12	91±9	80±7	1339± 109
Undisturbed							1257± 205

Table 5-7 The LH/CG (units/g ± s.d., n=4) and progesterone (P4)(ng/g ± s.d., n=4) secretion with (+P4) and without (control) exogenous progesterone (50ng/culture) at 2 stages of pregnancy (day 70 (a) and 80 (b)). In day 70 cultures, the media was completely renewed at +4 hours, a control sample was taken 2 hours later (+6 hours) and media either with or without progesterone was added. The day 80 placenta was cultured continuously with or without added progesterone.

(a) Day 70	Control (+ 6 hrs)	day 1	day 2	day 3	day 4	Total
LH/CG						
control	4000± 283	20212± 1020	6427± 458	2954± 226	2065± 260	31658
+P4	3800± 312	6669± 540	3654± 271	1855± 145	1401± 100	13579
P4						
control	30±2	50±9	30±7	15±6	10±4	105
+P4	28±2	47±8	32±6	17±3	11±2	106

(b) Day 80	day 1	day 2	day 3	day 4	Total
LH/CG					
control	1100± 5	825± 150	528± 59	800± 95	3253
+P4	1000± 100	1030± 148	597± 63	780± 80	3437
P4					
control	142±21	66±6	47±9	24±9	279
+P4	160±20	70±8	46±8	22±7	298

Table 5-8 The effect of exogenous pregnenolone (Preg) (70ng/culture) on the progesterone secretion (ng/g ± s.d., n=4).
(a) 110 days: The media was removed and renewed at +4 hours. 2 hours later (+6 hours) a control sample was taken (0.3ml) and the media was replaced (0.3ml) with or without added pregnenolone. The percentage difference between the treatment and control cultures is given for each day.
(b) 130 days: An 0.3ml control sample was taken 2 hours before the complete daily media change on day 2 and 4. 0.3ml of media with or without pregnenolone was added. The percentage increase for the 2 hour interval is given.

(a) 110 days

	Control + 6 hrs	Day 1	2	3	4	5
Preg.	152±43	900±19	400±19	340±50	140±15	100±20
Cont.	141±63	795±20	280±42	150±30	60±30	40±10
Sig(P)	N.S.	<0.05	<0.02	<0.01	<0.01	<0.01
% increase	7	13	43	126	217	150

(b) 130 days

Day 2

	Control +46 hrs	+48 hrs	% increase
Preg.	256±18	582±25	277±30
Cont.	410±90	569±40	138±40
Sig.	P<0.02	N.S.	P<0.01

Day 4

	Control +94 hrs	+96 hrs	% increase
Preg.	44±13	76±10	173±12
Cont.	62±10	72±12	116±10
Sig.	P<0.02	N.S.	P<0.02

amount of progesterone secreted from day 1 to 5 (a) was significantly greater when pregnenolone was added. Control samples showed no significant difference between the groups prior to treatment. The short term effect (2 hours) was examined in 130 day placental cultures (b). Although there was no significant difference in the final progesterone secretion between the treatment and control group either on day 2 or 4; the control group was secreting significantly more progesterone prior to the initiation of treatment. Therefore, the amount and percentage of increase was significantly greater for the treatment group.

Additional treatments which showed no effect on progesterone secretion either over the short term (2 hours) or long term (24 hours) were the addition of exogenous cholesterol (250ng/ml) and hCG (dosages 0.01 to 1000 i.u. per culture). hCG was tested between days 60 and 140. Antibodies to hCG, raised in the marmoset (Hearn, 1978) also did not affect progesterone secretion.

Culture media with these exogenous substances added gave undetectable results in the progesterone assay.

Placental tissue was also cultured in the presence of adrenal or gonadal tissue in cultures at 90-140 days of gestation. The oestrogen secretion from these cultures was compared with the control cultures. Oestrogen secretion from placental tissue cultured with either adrenals or testis was always greater than the controls. The percentage increase ranged from 180 to 720%. There

was no relationship between the percentage increase and the stage of gestation. There was no difference in the oestrogen secretion from placental tissue cultured with fetal ovaries. Androstenedione and testosterone were present in considerable amounts in cultures in which there was adrenal or testicular tissue. The amounts were not quantified per gram of tissue, as the weight of the cultured organ was not taken. Androgens were generally not measurable in the control placental cultures.

5.4 Discussion

This study comprises the first report of ovarian and placental function in the marmoset monkey. This was studied both *in vivo* and *in vitro*. It is the first study in any primate species in which ovarian and placental function has been assessed for several parameters in an integrated investigation throughout pregnancy. In view of the fact that the whole of pregnancy was studied, numbers at any one stage were limited. Interanimal variation could possibly indicate or obscure trends which more data at a specific stage would clarify. However, this drawback was minimised by the trends being fairly definitive, by the maximum interval between samples being 10 days (all timed pregnancies), by additional samples being taken at some stages, by serial sampling for UOV samples and by the interrelating of data from the various compartments. Furthermore, the use of the techniques chosen did not compromise further successful pregnancies which was an

important aspect, as larger numbers of animals could be used without drastically affecting the colony's breeding. As seen in the following discussion, the techniques chosen were able to define ovarian-placental function and relate it to a known gestational age.

Measurement of progesterone, oestradiol and oestrone in the UOV throughout pregnancy, and the relationship of the UOV levels to the presence or absence of CL and feto-placental unit, and to the MPV, was used to assess ovarian-placental function *in vivo*. Unfortunately, it was not possible to obtain uterine vein blood for comparison with the UOV, which would have given a more precise estimation of the relative ovarian and placental contributions.

(a) Early pregnancy: CL function

Higher UOV progesterone, oestradiol and oestrone levels than peripheral levels indicated ovarian and/or placental secretion of these hormones during pregnancy. The order of importance of the 3 hormones in quantitative terms in the UOV until day 80 was progesterone, oestrone and oestradiol, the same order as found in the MPV (Chapter 4).

The lack of a difference in the LH/CG level between the UOV and MPV in the samples measured was surprising in view of the high placental content and secretion *in vitro*. A similar finding was also reported for the rhesus monkey (Walsh *et al*, 1977b) and it may be

related to a considerably longer half life of this hormone as opposed to the steroid hormones.

(i) Progesterone and oestrone

The CL was the primary source of progesterone and oestrone after ovulation and for at least the first 50 days of pregnancy. Levels were elevated following ovulation. Significantly higher levels were associated with the presence of the CL up to day 50 of pregnancy whereas similar values were found in paired veins if both were associated with CL. Also, prior to day 50, the post/pre f.p.% was greater than 90%.

Higher UOV than MPV values were also found in the vein not associated with the CL. This may be due to vascular connections between the ovaries possibly via the uterine circulation (Riesen, Koering, Meyer and Wolf, 1970) that were not sufficiently clamped. The marmoset ovary is extensively luteinised (Hampton and Taylor 1971) which may also contribute to hormone levels.

Declining CL function between 20 and 60 days of pregnancy was indicated by declining UOV progesterone values. This trend was confirmed by samples taken at serial laparotomies. Furthermore, progesterone values declined only in the UOV associated with CL. In contrast, values in the contralateral UOV, which were not associated with a CL, increased slightly. The decline in the progesterone UOV values was not reflected in the MPV as seen in the declining UOV to MPV ratio. Peripheral levels may have reflected the initial decline in CL function as mean

levels declined between the 3rd and 5th week of pregnancy (Chapter 4). Thereafter, peripheral levels stabilised during the period of further declining CL function and of increasing placental progesterone production (see section 5.4b).

Conceivably, the dramatic decline in UOV progesterone values which occurred between days 20 and 60 could be attributed to changing blood flow which increases in the pregnant rhesus monkey (Lees, Hill, Ochsner, Thomas and Novy, 1971). Lower hormonal concentrations secreted from the placenta could be diluting the high UOV levels from the CL, resulting in a decline, and increasing the values in the vein not associated with the CL. To some extent, this may also account for the stabilisation of the maternal peripheral plasma levels after the 5th week of pregnancy. However, if this was the sole explanation, one might expect an earlier decline in the post/pre f.p.%, which in fact is not observed until day 60. Further studies are necessary to assess the effect of blood flow on the UOV values. Measurement of 17-hydroxyprogesterone, which is commonly used for the human (Yoshimi et al, 1969; Tulchinsky and Hobel, 1973; Mishell et al, 1973 and others) to reflect CL function during the first part of pregnancy, would help further define the decline in CL function.

A decline in CL function during early pregnancy has been well documented for both the human and rhesus. This was also not necessarily reflected in the peripheral plasma although a plateau or nadir in

progesterone values, during the period of declining CL function and prior to the full takeover of placental progesterone production, was often observed (Chapter 1 for references).

Between days 50 and 80, the CL was probably becoming less important in contributing to progesterone levels. Although levels still generally remained higher in the vein associated with the CL, the difference was not significant. Also the post/pre f.p.% gradually declined during this period. However, some ovarian contribution was indicated by the percentage remaining higher than that seen after 90 days of pregnancy.

A decline in CL oestrone secretion was less obvious than that seen for progesterone. That the trends were not of similar magnitude for progesterone and oestrone was reflected in the declining UOV progesterone to oestrone ratio. The oestrone trend may be partly obscured by the lower levels of oestrone and the greater interanimal variation in levels. A small placental contribution to oestrone levels may be more obvious than for progesterone and may partly account for the slightly lower oestrone post/pre f.p.% found for the first 50 days. Also, additional non-placental sources of oestrone may be important. This may obscure a decline in CL oestrone secretion and partly account for the longer persistence of the higher post/pre f.p.% for oestrone. In the human, oestradiol and oestrone are interconvertible and 15% of the oestradiol produced may return to the

circulation as oestrone (Longcope, Layne and Tait, 1968). Conversion from androstenedione is yet another source of plasma oestrone (McDonald, Rombaut, and Siiteri, 1967; Longcope, Kato and Horton, 1969).

(ii) Oestradiol

In contrast to progesterone and oestrone, UOV oestradiol values were not related to the presence or absence of the CL. In the human, during the normal menstrual cycle, the CL secretes oestradiol (Mikhail, 1970). Presumably in the marmoset, ovarian tissue other than the CL or extra-ovarian sources, contribute substantially to oestradiol levels. Follicles, which were often evident in early pregnancy (Hearn, J. unpubl'd data), may contribute to oestradiol levels, and preliminary observations in the marmoset, during the follicular phase, showed higher UOV oestradiol levels in animals in which developing follicles were evident. This may be a factor which obscured any relationship between the presence of CL and oestradiol levels and may also partly account for the marked difference in levels between the UOVs that was found during the first part of pregnancy.

(b) Placental Function

CL function during early pregnancy was discussed in section 5.4a. UOV data suggested that it was the major source of progesterone for the first 50 days of pregnancy; its importance thereafter declined until day 80 and it was relatively unimportant by day 90. A placental influence was becoming increasingly evident between 60 and 80

days as reflected in the declining post/pre f.p.% and the similarity in UOV hormonal levels.

The gestational changes, in the placental progesterone content and the secretion in vitro, reflected the proposed increases in placental function. There was a gradual increase in the placental progesterone content and total secretion between days 40 and 80. which can be attributed primarily to placental growth during this period, as the concentration and secretion per gram of tissue did not increase.

In contrast to progesterone, the placental concentration of oestradiol and oestrone increased between 40 and 80 days of pregnancy;therefore, the increase in total content was greater for these 2 hormones than for progesterone. In addition, lower amounts of oestrogens than progesterone are produced by the ovary in early pregnancy and therefore, the placental secretion may cause a greater percentage increase for oestrogen than for progesterone.

By 90 days of pregnancy, the placenta was undoubtedly the major source of progesterone and oestradiol, and was responsible for the steep increases which occurred in the UOV and MPV. This was evident by the steep decline in the post/pre f.p. %. In addition, the placental concentra-

tion and secretion per gram of tissue increased markedly, and combined with placental growth, resulted in a steep increase in the total amount of hormone in, and secreted by, the placenta.

The relationship between ovarian-placental function and the hormonal profiles in the maternal plasma (Chap. 4) show some similarities to the human (see Chap. 1 for human references). In both species, placental steroidogenesis is becoming established during the period of the highest peripheral CG levels, of relatively stable progesterone levels, and gradually increasing oestradiol levels. After completion of the luteo-placental shift, peripheral progesterone and oestradiol levels increase rapidly. Also, the more rapid increase in hormonal levels due to the increasing placental function occurs soon after completion of the embryonic period of development. (Phillips, 1976; See also Chap. 6.4). Differences from the human are that the decline in peripheral CG in the marmoset is more advanced when the more rapid increase in progesterone and oestradiol are initiated. Also, the increased placental secretion of these hormones occurs later in gestation in the marmoset (~66% of pregnancy completed) than in the human (~25% of pregnancy completed).

The declining progesterone and increasing oestradiol values in the MPV, found at the end of gestation, may be related to changes in placental secretion; as comparable changes were found in the UOV, the placental content and secretion in vitro. The factors responsible for

a change in placental function at this time, and the significance to the onset of labour, are unknown.

As seen above, an in vitro assessment of placental hormone content and/or secretion were related to the changing placental function with advancing gestation. This has not previously been shown for any species. If an in vitro placental culture system can be established and validated for a species (See section 5.3), it may then provide a good means for investigating factors influencing placental function.

Unlike the rhesus, there was no evidence for any recrudescence of CL function in the marmoset during late pregnancy. After 100-110 days of pregnancy, levels in the UOV, following removal of the feto-placental unit, were similar to those in the MPV immediately post-operative and to those in early pregnancy (less than 20 days) in the UOV not associated with CL. There was also no indication of an increase in the hormonal values in the UOV following removal of the feto-placental unit or of an increase in the post/pre f.p.%. These post removal levels were however higher than those in the follicular phase, and there may be some ovarian contribution persisting until the end of gestation, such as found in the human and rhesus (human: Mikhail and

Allen, 1967; Mikhail, 1970; Le Maire et al, 1970; Guraya, 1972; Crisp et al, 1973; Weiss and Rifkin, 1975. rhesus: Treloer et al, 1972; McDonald et al, 1973; Koering et al, 1973; Sholl et al, 1976; Walsh et al, 1974, 1979b). An ovarian contribution persisting until the end of gestation may also relate to the higher post/pre f.p.% found for progesterone and oestrone than found for oestradiol during the latter part of pregnancy. However, if there is any ovarian secretion, the funtion of such a low level of secretion in the face of the relatively massive placental secretion is not known. The renewal or continuance of CL function which occurs in the rhesus (loc cit) apparently extends into the post partum period (Weiss et al, 1973). This certainly does not appear to be true for the marmoset, and would seem to be incompatible with the immediate return to ovulatory cycles and fertility following birth, which occurs in this species.

However, a likely alternative explanation for the persistence of elevated post-removal hormonal values, would be a biphasic disappearance rate. The half-life of progesterone in pregnant macaques was 4 mintues for the initial phase of disappearance and about 30 mintues for the slower component of disappearance (Sholl and Wolf, 1974). Variations between the disappearance

rates of the hormones could account for the differences in their percentages.

Interpretation of hormonal values in the UOV following removal of the feto-placental unit must take account of certain possibilities. First, mentioned above, was the half-life of the hormones. However, the changes in each hormone's post/pre f.p.% are of such magnitude with advancing gestation that it seems unlikely to be the sole explanation and, in fact, for the human, the metabolic clearance rate (MCR) of progesterone, expressed as the biological half-life, is similar throughout pregnancy (Fylling, 1970b). Second, removal of the feto-placental unit undoubtedly changes the blood flow, probably to different extents during pregnancy. Third, the immediacy of a CL reaction to feto-placental removal is not known. It was assumed to be unaltered for 10 minutes following feto-placental unit removal, which was about the time it took for the UOV samples to be taken. This seemed likely in view of the greater than 90% post removal levels found in early pregnancy and was similar to results obtained in the human (Holmdahl, et al, 1971).

(i) The luteo-placental shift

Lutectomy experiments by Hearn (1978) suggested

that the CL was not dispensable until after the 6th week, which is nearly one-third the way through pregnancy. Although the present study did not define the stage of gestation at which the CL is actually dispensable, it did indicate that there is a substantial ovarian progesterone contribution and relatively little placental contribution for the first 50-60 days of pregnancy. It seems that the CL is important for relatively longer in pregnancy than in the human or rhesus. In the human, it is dispensable about one-seventh (Csapo et al, 1972), and in the rhesus one-eighth, (Bosu et al, 1974) the way through pregnancy. This is yet another aspect in which the events occurring during early pregnancy in the marmoset are prolonged compared to other species.

The present study indicated that at the 40th day (approximately 6 weeks) of gestation, there was still a substantial ovarian progesterone contribution and relatively little placental contribution, whereas Hearn (1978) suggested that the luteo-placental shift occurred around this time. Several explanations are possible for the apparent discrepency. Lutectomy is not entirely satisfactory in the marmoset as it is difficult to ensure removal of entire CL, and the ovary seems to contain extensive areas of luteal-like tissue (Hampton and Taylor, 1971). In fact, lutectomy in this species during the early luteal phase did not result in non-luteal phase progesterone values until more than 10 days following the operation (Hearn, J. unpub'd observations). The exact timing of the dispensibility of the CL would need to be confirmed by

ovariectomy on timed pregnancies.

However, it may be that the timing of the luteo-placental shift can preceed the predominance of progesterone production by the placenta (Holmdahl et al, 1971) and/or that the placenta may assume a relatively greater production of progesterone earlier than normal if the ovarian source declines earlier. Normal inter animal variation in the timing of increasing placental function or an earlier than 'normal' onset of increased placental function due to undefined causes may be suggested by the data from day 60 pregnancies. A comparatively low post/pre f.p.% was associated with a relatively high placental content of progesterone. UOV progesterone values for this animal were similar to others at this stage of gestation; however, a larger percentage was perhaps due to a placental contribution. It is not known whether the placenta can assume a greater proportion of progesterone production slightly earlier in pregnancy than 'normal' if for instance, the ovarian source declines. Presumably the placenta must be developed sufficiently for steroidogenesis to increase which no doubt puts a time limitation in pregnancy during which this can occur. Also, there must be some stimulus or feedback relationship. The idea of a feedback relationship between the placenta and ovary is not new, but it was postulated to work in the opposite direction (Lanman et al 1975). In fact, little work has been done on ovarian-placental interrelationships. The marmoset may be a good subhuman species for such studies, as the 'transitional'

period of ovarian placental function has been well defined in the present study. (See also Chap 8).

(c) Placental hormonal content

The changes in progesterone, oestradiol and oestrone placental values have already been discussed in relation to ovarian and placental function (section 5-4b). Interspecies comparison of the placental content of progesterone shows that fairly comparable amounts are found in man (Zander and von Munstermann, 1956; Runnebaum et al, 1975a and b), chimpanzee (Hobson, 1971) and marmoset; and much lower amounts in the rhesus (Short and Eckstein, 1961; Hagemenas and Kittinger, 1974). This corresponds to the variation in peripheral hormonal values found between these species (Chapter 1 for references).

However, the pattern of progesterone concentration with advancing gestation in the human is different from the marmoset in that higher or similar placental concentrations are found during the first half of pregnancy rather than the second. Whereas it was due solely to substantial placental growth that the total placental progesterone content increased in the human during the period of increasing peripheral levels, it was due both to placental growth and increased concentrations in the marmoset. The reason for the difference is not known. The relative placental to fetal weight ratio is lower in the marmoset than in the human (see Chapter 7 for data and references), and it may be advantageous for a small primate which

regularly produces more than 1 young but requires high hormonal levels, to minimise relative placental size.

Placental concentrations of LH/CG, androstenedione and testosterone were compared only with peripheral plasma levels. The growth of the placenta during the first half of pregnancy modulated the steep decline in the placental concentration of LH/CG. Nevertheless, peripheral concentrations were still increasing steeply (between 30 and 50 days; Chapter 4) when the placental content of LH/CG was already in the uppermost part of the range. This could perhaps be due to an increasing secretion rate due to an increasing blood volume through the tissue. By 100 days of gestation the low amounts of placental LH/CG were reflected in the periperal plasma. The placental LH/CG concentration shows an inverse pattern to progesterone or oestradiol. Any further relationship must await more dynamic studies as have been recently pursued (Wilson et al, 1980b; see Chapter 1).

The pattern of the androgens in the placental tissue was different from that in the MPV. The higher placental androgen levels found in the latter part of pregnancy were not reflected in the MPV. Other sources of these hormones (e.g. adrenal and ovary) or peripheral conversions may be more significant to MPV levels than a placental contribution (Horton and Tait, 1966). The effective aromatase system of the placenta (Ryan, et al, 1961)

may also serve to protect the maternal compartment from receiving large amounts of placental androgens. Although measurement of placental content gives no idea of placental utilisation of these hormones, the increase in androgens in the placental tissue coincids with the increased oestrogen placental content and MPV levels. The marmoset placenta, in common with that from several other primate species, utilises C_{19} substrates for oestrogen production; all being unable to utilise C_{21} precursors (reviewed; Ryan and Hopper, 1974).

Hormonally, there was no evidence for a major and minor placental disc, as in the majority of instances each placental disc contained similar amounts of hormones. Either each disc produced similar amounts, or placental anastomoses abolished any differences. An explanation for the difference in hormonal values found in some instances is not available. The methodology did not appear to give a false result and there was no consistent association between hormones. In other species, fetal sex may affect placental hormone production and gonadotrophin content (Hagemenas and Kittinger, 1973, 1974; Hobson and Wide, 1974). Support for this idea, or even just the presence or absence of a fetal attachment, was not definitive in the present study. This is perhaps not surprising in view of the considerable overlap observed in other species (loc cit). There did not appear to be a relationship, but analysis in the present study was complicated by the occurrence of singleton, twin and triplet pregnancies and

that fetuses were attached to either one or both discs (Chapter 7). Gestational changes, with the fetal effect more pronounced at one stage than another, may further complicate the matter.

(d) Placental hormone secretion in vitro

Comparison of the secretion of progesterone by placental tissue maintained in organ culture with the stage of gestation supported the suggested pattern of relative ovarian and placental function, and has been discussed in section 5.4b.

The secretion of progesterone in vitro was similar overall to the progesterone content in the tissue. A similar finding was made for the human placenta in that Wilson and Jawad (1980) found that human placental tissue from late gestation spontaneously produced less progesterone in the media than tissue from early pregnancy, a similar pattern to the content.

The secretion of LH/CG was similar to the peripheral hormonal pattern and the placental content, with maximum secretion during the first half of gestation followed by a decline to low or undetectable levels during the latter half. Undetectable levels of LH/CG in the culture media during the latter half of gestation compared to

measurable amounts in the placental tissue was probably due to the dilution of any LH/CG secreted by the placental tissue to below the detection limit of the assay by the volume of culture media used.

The pattern of LH/CG and progesterone secretion observed in this study with successive days of culture is similar to that reported by other authors also utilising organ culture for human placental tissue (Golander, Barrett, Tyrey, Fletcher and Handwerger, 1978; Huot, Foidart and Stromberg, 1979; Wilson et al, 1980 ; Wilson and Jawad, 1980). This is a pattern of declining progesterone secretion, and an initial decline in hCG secretion followed by an increase.

All of the studies have provided evidence of in vitro hormonal synthesis by either comparison of the amount of hormone secreted in culture compared to that in the placental tissue, conversion of radioactive or non-radioactive precursors to progesterone, ^{3}H leucine incorporation into hCG, or stimulation of hCG by agents such as 3'5'dibutyrylcyclic AMP. Inhibition of hormone secretion by agents such as cyclohexamide and aminoglutethimide has also been shown. Golander and associates (1978) suggested that hCG released into the medium during the first 24 to 48 hours was almost all preformed and that the increase in hCG secretion after 3-4 days of culture was due to the disappearance in time of an inhibitory factor on hCG synthesis that was present at the time of placental removal (Golander et al, 1978). Huot and coworkers (1979) are exploring the relationship to DNA synthesis and the pattern

of hCG secretion observed *in vitro*.

The steepest increase in the progesterone secretion *in vitro* occurred during the first 4 hours of culture and quite possibly represented a release of preformed steroid, some possibly due to tissue damage by the processing procedure. A continual decline in the progesterone secretion with successive days in culture was possibly due to the lack of a factor which maintains steroidogenesis *in vivo*. This factor did not appear to be cholesterol, which was available in high concentrations in the culture media (T. Baker, pers. comm.) and additional cholesterol did not boost secretion. Progesterone secretion also was not affected by exogenous progesterone or by media renewal. Pregnenolone did boost secretion, and this may be a limited precursor *in vitro*.

Despite changes with successive days of culture of what the secretion *in vitro* probably represents, e.g. from a degree of preformed hormone to increasing *in vitro* synthesis; the gestational changes in LH/CG and progesterone were seen throughout the culture period.

The culture system was primarily employed to compare the secretion of LH/CG and progesterone by placental tissue *in vitro* with the stage of gestation and to relate its its in vitro function to the results obtained in vivo and with the placental content. This aspect was fulfilled. It was also desired to assess its capabilities

as a system for investigations of the factors regulating hormone secretion. Experimentation with a few possible factors met with little success. This may be that the treatments tried would really have no effect on placental hormone secretion. There is no concrete evidence to suggest that there is an interrelationship between progesterone and CG. The inverse relationship between the 2 hormones that is found in several species (Chapter 1) and the occurrence of recurrent abortions in animals immunised against βhCG during 4 to 10 weeks of gestation when placental steroidogenesis was presumably being established in the marmoset (Hearn, 1976), initially led to the investigation between the 2 hormones. The lack of an effect may also be due to the dosages used (although a wide range was tried), or to the lack of significant biological effect on marmoset placental tissue of a human CG preparation and of an antibody, which although raised in the marmoset, was an antibody to human CG. Marmoset CG is not yet available.

In addition to pregnenolone, the only positive result in the regulation of hormone secretion was possibly the depression of LH/CG secretion at 70 days of gestation with the addition of exogenous progesterone. However, attempts to repeat this at a slightly later stage of gestation were unsuccessful. That there may well be such an effect on LH/CG production by progesterone was recently shown by Wilson et al, (1980). The effect seemed to be dose-dependent. The difference between the 2 experiments in the present study may well be related to the dosage of

progesterone used compared to the LH/CG secretion, and/or to a change in the sensitivity of the tissue to exogenous progesterone with the change in gestational age. LH/CG secretion *in vitro* had declined sharply by 80 days of pregnancy.

This lack of correlation in the results from the 70 and 80 day placenta highlighted a problem in the present set up for investigating hormonal regulation. Samples were taken from different stages of gestation due to the other requirements of the study. This will no doubt affect the responsiveness of the tissue to various treatments and possibly the dosages needed. It was also necessary to use several sets of culture to define the optimal culture conditions, the hormonal patterns with advancing time, the testing regimes and to validate the system. Marmoset placental tissue may not be suitable for extensive organ culture studies during the first half of pregnancy due to the small amount of tissue available (<2 grams), the high interculture variation in hormone secretion, the low progesterone secretion prior to day 80 and the low LH/CG secretion after day 90.

The secretion of oestradiol from placental tissue *in vitro* was increased when the placental tissue was cultured either with adrenal tissue or gonadal tissue (testis only). This result ties in with previous studies in the iris monkey (Davies *et al*, 1970) and the observation that the marmoset placenta utilises C_{19} precursors for placental oestrogen production (Ryan *et al*, 1961). It would be of interest to know if the marked increase in

placental oestrogen production at 90 days is related to an increased availability of fetal adrenal precursors. Although this increase in placental oestrogen (and progesterone) secretion occurs relatively later in gestation in the marmoset than in the human, it occurs at a similar stage of fetal development (See also Chapter 6-4); and the findings in this thesis showed that the marmoset fetal adrenals and testis secreted large quantities of androgen *in vitro* after day 90.

5.4 Chapter summary

1) Corpus luteum and placental function were assessed throughout pregnancy by a) hormonal measurements in the paired utero-ovarian vein, and their relationship to the presence or absence of the corpus luteum and of the feto-placental unit, b) the placental hormonal content and c) the placental hormone secretion *in vitro*.

2) Progesterone and oestrone values were related to the presence or absence of the corpus luteum in early pregnancy, whereas oestradiol values were not.

3) Corpus luteum function declined between days 20 and 60 with the steepest decline between days 40 and 60. It makes little contribution to hormonal values by day 90 of pregnancy.

4) The placenta is an insignificant source of progesterone and oestrone prior to day 50. Between days 50 and 80, there is a transitional period from ovarian to placental dominance. By day 90, the placenta is definitely the major

source of progesterone and oestradiol.

5) The trends for the placental content of progesterone, oestrogen, and LH/CG were similar to those in the maternal peripheral plasma, whereas the androgen placental content profile was distinct from the maternal profile. Placental androgen content is increased coincident with the increased oestrogen placental content.

6) A placental organ culture system was validated and utilised for measurement of endogenous hormone secretion. It appeared to reflect the relative capacity of the placenta for progesterone and LH/CG production at different stages of gestation.

7) The placental hormonal content is generally similar in each placental disc.

CHAPTER 6 : FETAL HORMONES

6.1 Introduction

Fetal hormones, some of which show a sex difference, may be important for fetal maturation (possibly the onset of labour), sexual differentiation and placental hormone production). These topics have been extensively reviewed for the human and rhesus (Jost 1970a and b; Reyes, Winter and Faiman, 1976; Winter, Fairman and Reyes, 1977; Resko, 1977; Beling, 1977; Zondek and Zondek, 1979.)

The marmoset is unusual among primates in that it regularly produces dizygotic twins. There is apparently an early devlopment of a shared placental circulation and hematopoietic chimerism, but females born cotwin to a male are normal (Wislocki, 1939; Benirschke and Layton, 1969). Although anatomical sexual differentiation occurs prenatally, the extent to which brain or behavioural sexual differentiation occurs prenatally is not known. For the first time for a primate species, a post-natal influence was indicated by the study of Abbott (1979). Whether in this respect, the marmoset is unique among primate species is yet to be established.

In view of these unusual characteristics of the marmoset, it was of considerable interest to define the hormonal environment in which male and female marmoset fetuses develop normally. This study was designed, in conjunction with the studies outlined in Chapter 5.

to delineate the fetal hormonal changes occurring with advancing gestation and to compare hormonal values in male and female fetuses. Measurement of several hormones and multiple sampling sites (e.g. peripheral plasma, umbilical vein plasma, fetal plasma and amniotic fluid) allowed intercompartmental comparisons of the hormonal values and ratios. Hormonal values were also related to placental function which was reported in Chapter 5. In conjunction with other studies which utilised material from these fetuses, the fetal hormonal levels were also compared with fetal gonadol development and fetal gonadol and adrenal secretion *in vitro*.

6.2 Procedures

Fetal samples were obtained following hysterotomy. (Chapter 2.6). The sampling regime is given in Chapter 2.7.

Amniotic fluid was collected after day 70 of pregnancy using a 21-25 gauge needle. The fluid volume was estimated. Fluid was not always obtainable, because the sac ruptured on removal from the uterus, particularly in late pregnancy.

The umbilical cord was clamped at the fetal side and blood was taken from the umbilical vein after day 90 using a 21-27 gauge needle. No umbilical arterial blood was taken due to the small size of the artery. Fetal plasma was obtained after day 90 by cardiac puncture using a 21-27 gauge needle.

All blood samples were centrifuged and stored at -20°C until assayed (Chapter 2.3). Amniotic fluid was stored at -20°C until assayed.

6.3 Results

(a) Samples

Details of the number of pregnancies, the fetal sex and the samples obtained for fetal and umbilical vein plasma and anmiotic fluid are given in Table 6-1, Maternal plasma was taken from all pregnancies. Amniotic fluid was also obtained at day 70 but the fetal sex was not determined. The volume of amniotic fluid obtained ranged from 0.06ml at day 70 to 4ml at day 110. Small sample volumes often precluded the measurement of all hormones. There were more female than male fetuses and after day 90, 4 pregnancies had only female fetuses (1 or 2), 2 had female and male co-twins and 4 had 2 female and 1 male fetus.

(b) Hormonal values

(i) Progesterone

Figure 6-1 shows the individual progesterone values in the maternal fetal and umbilical vein plasma and in the amniotic fluid. Table 6-2 gives the mean value for each pregnancy and the mean value before and after day 90. Progesterone was measured in the fetal plasma only after 100 days of pregnancy. In the other 3 compartments, there was a severalfold increase in progesterone values between 90 and 100 days of pregnancy. Maximum

Table 6-1 The number (No.) of female (F) and male (M) fetuses and the samples obtained for the fetal serum (F.S.) umbilical vein (U.V.) and amniotic fluid (A.F.) between 80 and 140 days of pregnancy. The overall total and total from 100-140 days is also given.

Day		No.		F.S.		U.V.		A.F.	
		M.	F.	M.	F.	M.	F.	M.	F.
	80	1	1	0	0	0	0	1	1
	80	1	1	0	0	0	0	1	1
	85	1	1	0	0	0	0	1	1
8W	90	1	2	1	0	0	1	1	2
57W	90	1	2	0	0	1	2	1	2
	100	1	1	1	1	1	1	1	0
	100	0	2	0	2	0	0	0	2
	105	1	2	1	2	1	2	1	2
	110	0	2	0	2	0	2	0	2
	115	0	1	0	1	0	1	0	1
	120	1	2	1	2	1	2	1	2
	130	1	1	1	1	1	1	1	
	130	1	2	1	2	1	2		1
	140	1	2	1	2	1	2	0	0
28W	140	0	2	0	2	0	2	0	0
Total									
(day 80-140)		11	24	7	17	7	18	9	18
(day 100-140)		6	17	6	17	6	15	4	10

Fig. 6-1: Progesterone values (ng/ml) in the maternal peripheral vein (M.P.V.) (○△), amniotic fluid (A.F.), fetal serum (F.S.) and umbilical vein (U.V.) in females (○,△) and males (●,▲). Circles (○,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation.

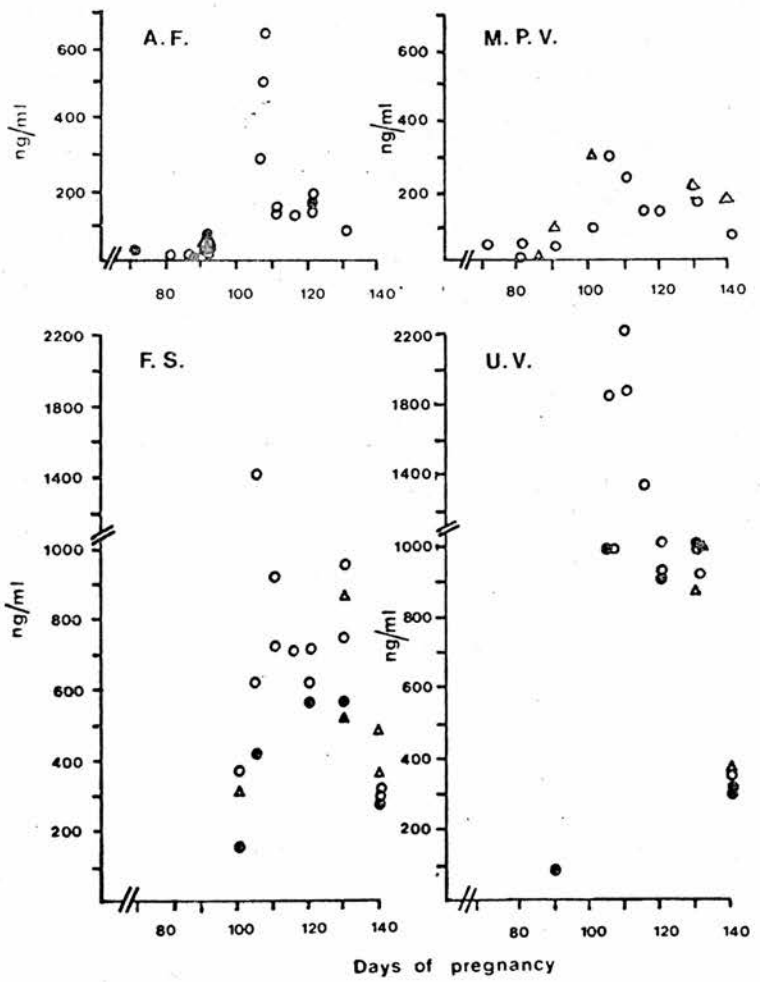


Table 6-2. Mean progesterone values (ng/ml) in the fetal serum (F.S.), umbilical vein (U.V.), amniotic fluid (A.F.) and maternal peripheral vein (M.P.V.). The number in parenthesis gives the number of samples included in each mean value. Only one M.P.V. sample was taken. The mean value before and after 90 days of gestation is also given.

Anim. No.	Stage of Gest.	F.S.	U.V.	A.F.	M.P.V.
28W	140 T	421 (2)	382 (1)		196
60W	140	301 (3)	339 (3)		97
33W	130	754 (3)	978 (3)	104 (1)	190
34W	130 T	712 (2)	878 (2)		289
10W	120 T	633 (3)	956 (3)	174 (3)	156
11W	115	707 (1)	1352 (1)	144 (1)	167
23W	110 T	827 (2)	1997 (2)	152 (2)	255
24R	105 T	844 (3)	1285 (3)	485 (3)	311
6R	100 T	312 (1)		94 (2)	118
44W	100	375 (1)			
57W	90 T		104 (2)	48 (2)	54
8W	90 T		93 (1)	28 (3)	63
51W	85 T			32 (2)	56
46W	80			22 (2)	70
21R	80 T				
48W	70 T			37 (1)	65
38R	70				
Mean 100-140 (±s.d.)		588±214	1020±538	192±146	197±74
Mean 70-90 (±s.d.)			98	33±10	62±6

fetal, umbilical vein and amniotic fluid values were found between 105 and 130 days of pregnancy. Fetal and umbilical vein values at 140 days were similar to those found at 90 to 100 days of pregnancy.

If all the data are considered, female and male fetal serum progesterone values are not significantly different from each other. However, in 6 pregnancies in which there was both a male and female fetus (gestation range 105-140 days), progesterone levels in the female fetus (mean = 636 ± 100ng/ml, s.d.) always exceeded those in the male fetus (479 ± 75ng/ml, s.d.). The difference was significant ($p<0.02$; paired t-test). There was no significant difference in the umbilical vein values in these pregnancies. Table 6-3, which gives the umbilical vein-fetal serum difference, shows that in 4 of these 5 pregnancies, the values were lower for the female than the male, and similar between female co-twins. In all cases, umbilical vein values exceeded fetal serum values although the difference decreased as pregnancy advanced ($p<0.001$, paired t-test)

(ii) Oestradiol

The individual oestradiol values in the maternal fetal and umbilical vein plasma are shown in Figure 6-2, and the mean values for each pregnancy are given in Table 6-4.

Amniotic fluid and umbilical vein oestradiol values were low prior to 90 days of pregnancy. Levels increased gradually after 90 days and maximum

Table 6-3. The difference (ng/ml) in the progesterone value between the fetal serum (F.S.) and umbilical vein (U.V.). A negative number indicates a higher value in the U.V.

Day of Gestation	Anim. No.	Female ng/ml	Male ng/ml
105	24R	-387	-565
		-368	
110	23W	-1197	
		-1142	
115	11W	-645	
120	10W	-310	-349
		-310	
130	34W	-1	-336
130	33W	-56	-430
		-185	
140	60W	-35	-32
		-48	
140	28	-25	

Fig. 6-2: Oestradiol values (ng/ml) in the maternal peripheral vein (M.P.V.)(○,△) , amniotic fluid (A.F.) fetal serum (F.S.) and umbilical vein (U.V.) in females (○,△) and males (●,▲). Circles (○,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation.

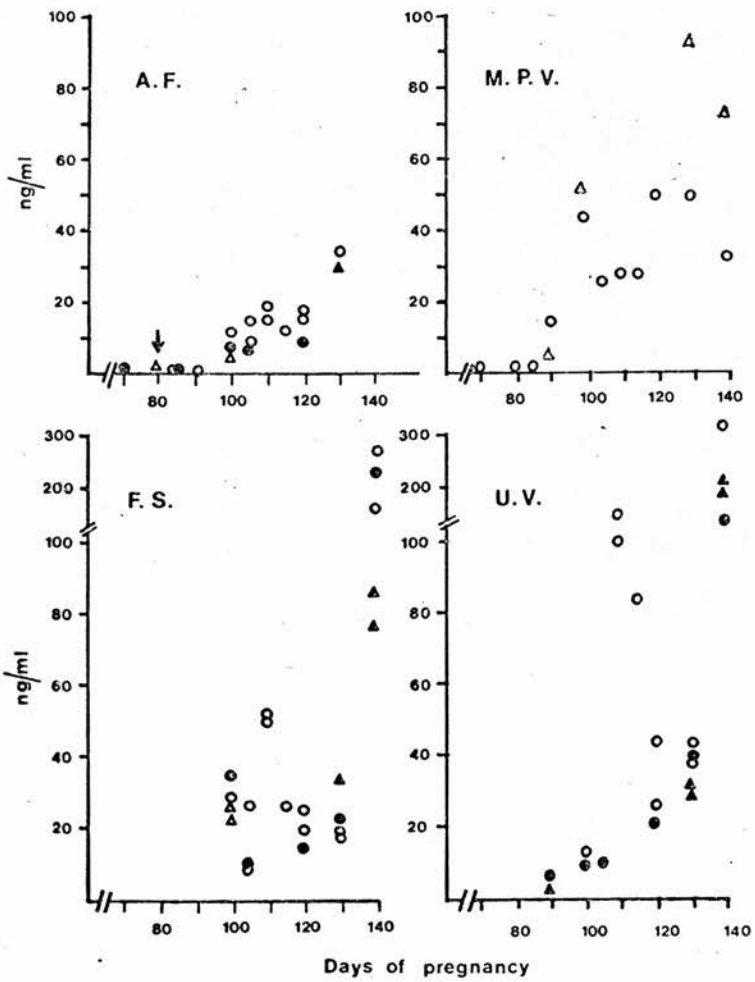


Table 6-4. Mean oestradiol values (ng/ml) in the fetal serum (F.S.), umbilical vein (U.V.), amniotic fluid (A.F.) and maternal peripheral vein (M.P.V.). The number in parenthesis gives the number of samples included in each mean value. Only one M.P.V. sample was taken. The mean values before and after 90 days of gestation are also given.

Anim. No.	Day of Gest.	F.S.	U.V.	A.F.	M.P.V.
28W	140	80 (2)	189 (2)		71
60W	140	218 (3)	310 (2)		131
33W	130	20 (3)	40 (3)	35 (1)	49
34W	120	28 (2)	29 (2)	30 (1)	92
10W	120	20 (3)	29 (3)	23 (3)	45
11W	115	26 (1)	82 (1)	17 (1)	49
23W	110	51 (2)	129 (2)	11 (2)	28
24R	105	16 (3)	10 (1)	10 (3)	25
6R	100	24 (2)		12 (2)	51
44W	100	32 (2)	10 (2)		43
57W	90		≤4.9 (2)	1.3 (3)	5
8W	90		1.1 (1)	0.9 (3)	14
51W	85			0.8 (1)	1.2
46W	80			<0.7 (1)	1.1
21R	80				3.7
48W	70				1.4
38R	70			1.1	1.4
Mean 100-140 (±s.d.)		52±62	81±77	19±11	58±32
Mean 70-90 (±s.d.)			3	0.9±0.3	4±4

values were found at 130-140 days. Fetal serum oestradiol values did not increase between 100 and 130 days, but like the umbilical vein levels, there was an approximately 6-fold increase between 130 and 140 days of gestation. Such a marked increase was not reflected in the maternal peripheral plasma.

There was no sex difference in oestradiol levels either overall or in pregnancies in which there was both a male and female fetus.

(iii) Oestrone

Oestrone values in the maternal, fetal and umbilical vein plasma and the amniotic fluid are given in Figure 6-3, and mean values for each pregnancy are given in Table 6-5. Levels in the maternal plasma were in the range generally observed for these stages of gestation (see Chapter 4). Although the highest maternal values were found in 2 pregnancies at 140 days, there was no general increase between 90 and 140 days. In contrast, oestrone values in the fetal serum, umbilical vein, and amniotic fluid showed a sustained increase after 90 days of gestation and maximum values were found at 140 days. The overall increase was approximately 10-fold between the 100th and 140th day of pregnancy in the fetal and umbilical vein plasma and about 25-fold between the 90th and 130th day in the amniotic fluid. Prior to 90 days, the amniotic fluid values were relatively low and constant.

Similar to oestradiol, there was no sex difference either overall or in pregnancies in which both a female and male fetus were present.

Fig. 6-3: Oestrone values (ng/ml) in the maternal peripheral vein (M.P.V.) (○,△), amniotic fluid (A.F.), fetal serum (F.S.) and umbilical vein (U.V.) in females (○,△) and males(●,▲). Circles (○,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation.

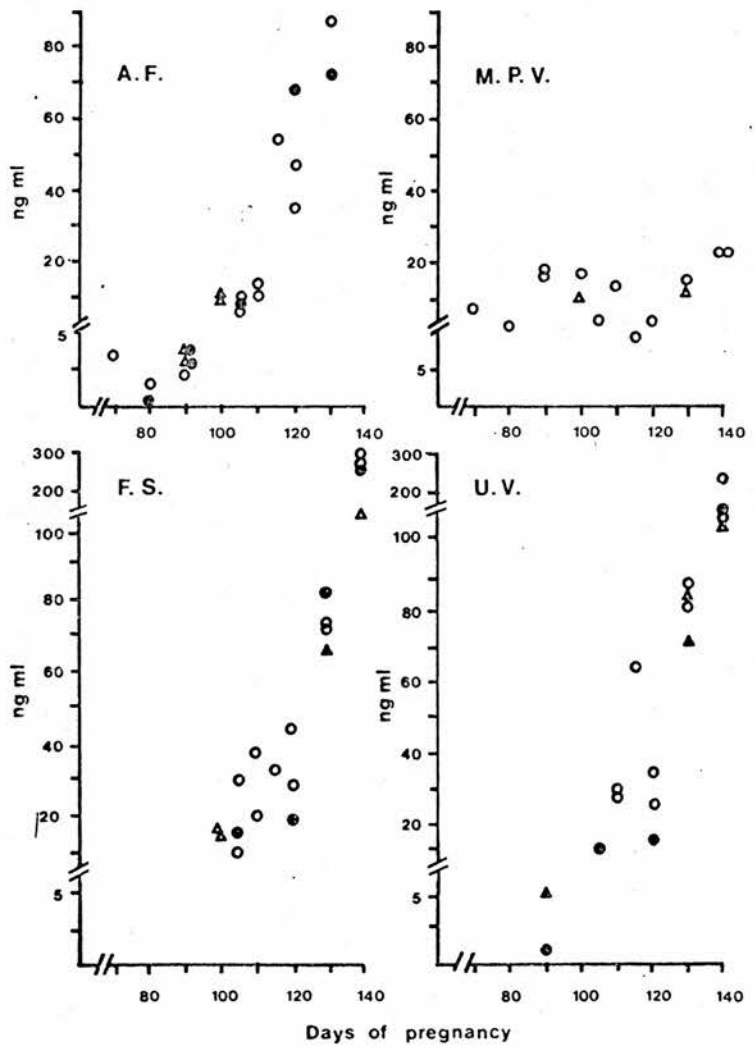


Table 6-5. Mean oestrone values (ng/ml) in the fetal serum (F.S.), umbilical vein (U.V.), amniotic fluid (A.F.) and maternal peripheral vein (M.P.V.). The number in parenthesis gives the number of samples included in each mean value. Only one M.P.V. sample was taken. The mean values before and after 90 days of gestation are also given.

Anim. No.	Day of Gest.	F.S.	U.V.	A.F.	M.P.V.
28W	140	185 (2)	130 (1)		23
60W	140	273 (3)	180 (3)		23
33W	130	74 (3)	83 (3)	89 (1)	15
34W	130	69 (2)	77 (2)	74 (1)	12
10W	120	31 (3)	25 (3)	51 (3)	7
11W	115	33 (1)	63 (1)	55 (1)	16
23W	110	24 (2)	28 (2)	14 (2)	15
24R	105	18 (3)	13 (1)	10 (3)	7
6R	100	16 (2)		13 (2)	11
44W	100				18
57W	90			3.2 (3)	19
8W	90			3.4 (3)	20
51W	85				
46W	80				
21R	80			1.6 (2)	7
48W	70			4.2 (1)	9
38R	70				
Mean 100-140 (±s.d.)		86±93	75±57	44±31	15±6
Mean 70-90 (±s.d.)				3±1	10±6

(iv) Androstenedione

Figure 6-4 shows the androstenedione values in the maternal peripheral, fetal and umbilical vein plasma and in the amniotic fluid. Table 6-6 gives the mean hormonal values for each pregnancy. Maternal values remained relatively constant between 70 and 140 days and were similar to those reported in Chapter 4. In contrast, the low androstenedione values found prior to day 90 were followed by a 2-3 fold increase after day 90 in the fetal serum, umbilical vein and amniotic fluid.

There was no sex difference in androstenedione values either overall or in the 5 pregnancies with both a female and male fetus.

(v) Testosterone

The maternal peripheral, fetal and umbilical vein plasma and amniotic fluid testosterone values are shown in Figure 6-5. Mean values for each pregnancy are given in Table 6-7. Prior to and including 90 days of pregnancy, testosterone levels were low in the maternal, fetal and umbilical vein plasma and in the amniotic fluid. Only 2 of the 9 70 to 90 day amniotic fluid samples and neither of the 90 day umbilical vein samples contained measurable testosterone ($>$0.2ng/ml). Between 90 and 100 days, amniotic fluid, fetal serum and umbilical vein levels increased and thereafter remained elevated.

There was no overall sex difference in fetal testosterone values and in fact, the highest fetal (and umbilical vein), testosterone values were found in a

Fig. 6-4: Androstenedione values (ng/ml) in the maternal peripheral vein (M.P.V.) (○), amniotic fluid (A.F.), fetal serum (F.S.) and umbilical vein (U.V.) in females (○,△) and males (●,▲). Circles (○,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation.

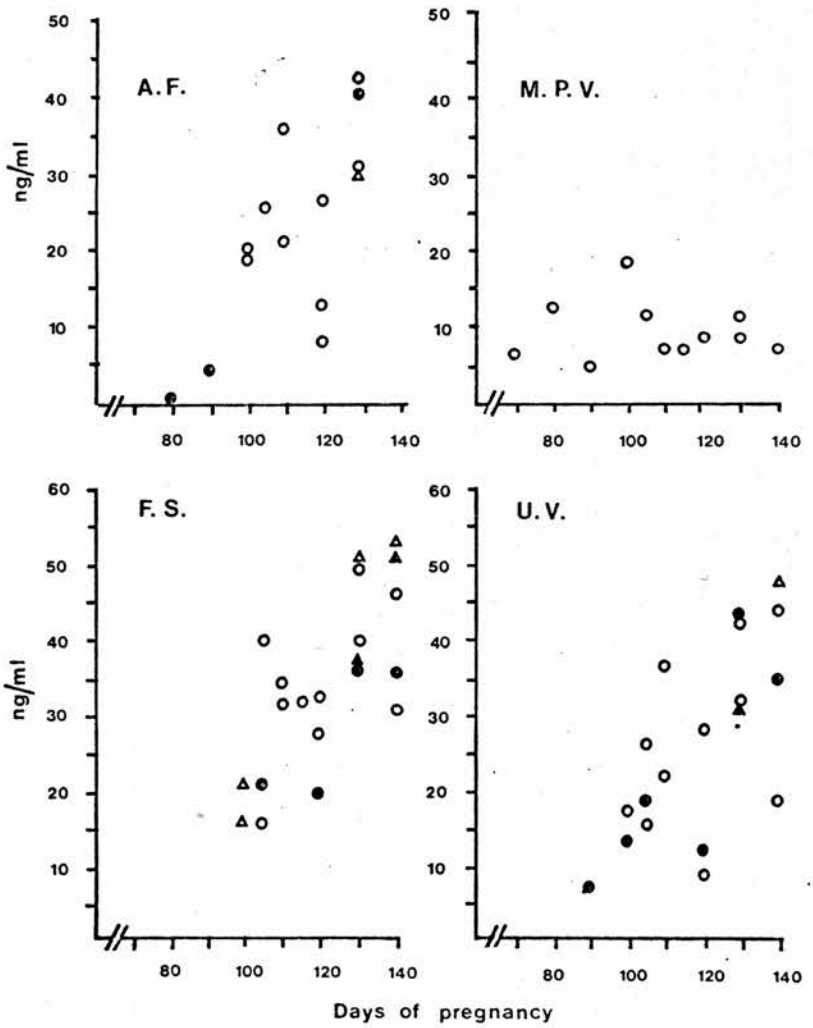


Table 6-6. Mean androstenedione values (ng/ml) in the fetal serum (F.S.), umbilical vein (U.V.), amniotic fluid (A.F.) and maternal peripheral vein (M.P.V.) The number in parenthesis gives the number of samples included in each mean value. Only one M.P.V. sample was taken. The mean value before and after 90 days of gestation is also given.

Anim. No.	Stage of Gest.	F.S.	U.V.	A.F.	M.P.V.
28W	140 T	51.6 (2)	47.6 (1)		8.71
60W	140	36.9 (3)	31.9 (3)		
33W	130	41.0 (3)	38.87 (3)	22.50 (1)	9.60
34W	130 T	43.27 (2)	30.88 (1)		12.0
10W	120 T	26.12 (3)	16.58 (3)	41.3 (2)	
11W	115	31.16 (1)		18.46 (1)	10.30
23W	110 T	32.44 (2)	29.86 (2)	29.20 (2)	7.43
24R	105 T	24.70 (3)	19.90 (3)	33.30 (3)	12.06
6R	100 T	17.80 (2)		19.27 (2)	6.02
44Q	100		15.06 (2)	18.93 (1)	19.31
8W	90 T		7.28 (1)	4.86 (1)	4.86
46W	80			0.81 (1)	13.71
38R	70			$<$0.50 (1)	7.43
Mean 100-140 (±s.d.)		33.89±10.45	28.76±11.27	26.13±8.78	10.67±4.06
Mean 70-90 (±s.d.)			7.28	$\leq$2.05±2.43	8.67±4.55

Fig. 6-5: Testosterone values (ng/ml) in the maternal peripheral vein (M.P.V.) (○△), amniotic fluid (A.F.), fetal serum (F.S.) and umbilical vein (U.V.) in females (○,△) and males (●,▲). Circles (○,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation. The arrows (↓) means testosterone was immeasurable (See Table 6.7)

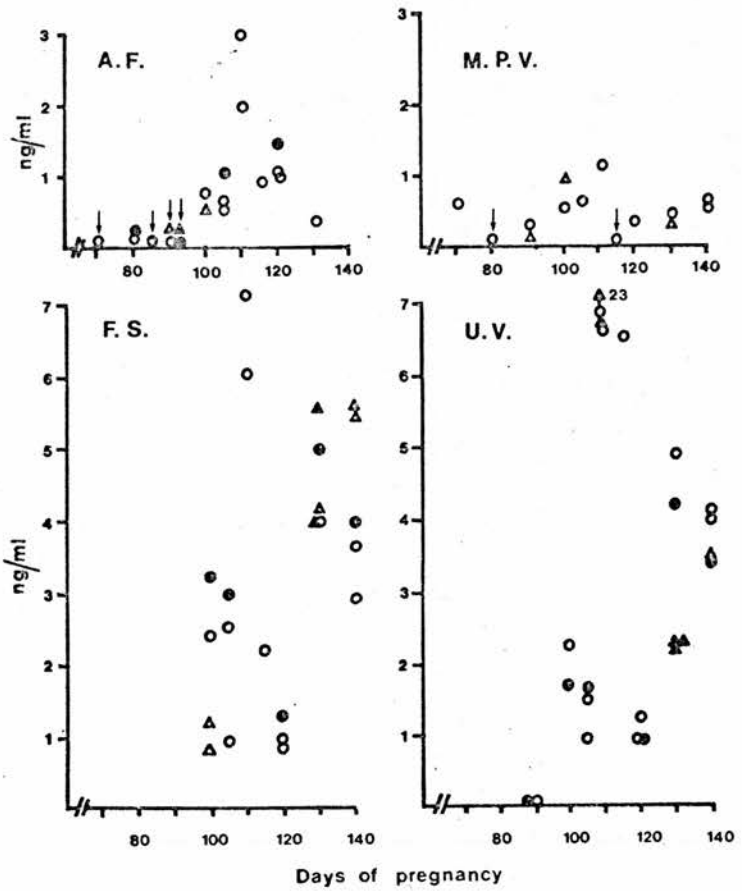


Table 6-7 . Mean testosterone values (ng/ml) in the fetal serum (F.S.), umbilical vein (U.V.), amniotic fluid (A.F.) and maternal peripheral vein (M.P.V.). The number in parenthesis gives the number of samples included in each mean value. Only one M.P.V. sample was taken. The mean value before and after 90 days of gestation is also given.

Anim. No.	Stage of Gest.	F.S.	U.V.	A.F.	M.P.V.
28W	140 T	5.31 (2)	3.61 (2)		0.71
60W	140	3.60 (3)	4.01 (3)		0.72
33W	130	4.60 (3)	2.27 (3)	0.44 (1)	0.50
34W	130 T	4.55 (2)	4.77 (2)	2.00 (1)	0.40
10W	120 T	1.08 (3)	1.66 (3)	1.21 (3)	0.44
11W	115	2.24 (1)	6.63 (1)	0.96 (1)	0.20
23W	110 T	7.56 (2)	16.89 (2)	2.50 (2)	1.22
24R	105	1.58 (3)	1.46 (3)	0.82 (3)	0.71
6R	100 T	1.01 (2)		0.78 (2)	1.03
44W	100	2.84 (2)	2.04 (2)	0.46 (2)	0.64
57W	90 T		<0.40 (2)	<0.20 (3)	<0.20
8W	90 T	<1.0 (1)	<0.40 (2)	< .20 (3)	0.24
51W	85			<0.20 (2)	0.50
46W	80			0.23 (2)	1.62
38R	70			<0.20 (2)	0.70
Mean 100-140 (±s.d.)		3.44±2.10	4.79±4.83	1.14±0.73	0.65±0.31
Mean 70-90 (±s.d.)		< 1.0	≤0.95±0.94	≤0.21±0.01	0.65±0.57

pregnancy with 2 female fetuses. These high levels were not reflected in the maternal peripheral plasma. However, in the 6 pregnancies in which both a female and male fetus was present, the male testosterone values were significantly higher than the closest values from the female counterpart ($P<0.025$, paired t-test). There was no corresponding difference in umbilical vein values. Even in these 6 pregnancies, there was overlap between males and females; with the female values ranging from 1 to 4.2ng/ml and the males from 1.3 to 5.5ng/ml.

Table 6-8 shows the umbilical vein-fetal plasma difference. In 5 of 6 instances, there was a larger positive difference (favouring the fetal compartment) for the male than the female counterpart within a pregnancy although overall the differences overlapped. Fetal serum testosterone values were significantly higher than umbilical vein values for male fetuses ($P<0.01$, paired t-test, $n=6$) but not for female fetuses. Male fetal serum values were always higher than umbilical vein values whereas female fetal serum values were both higher and lower.

(vi) LH/CG

LH/CG was measured in a few samples in which there was sufficient plasma. In all cases the LH/CG values were undetectable. The following stages of gestation were sampled: 90 to 120 days: amniotic fluid; 110, 130, 140 days: fetal plasma; 120, 130, 140: umbilical vein plasma.

Table 6-8. The difference (ng/ml) in the testosterone value between the fetal serum (F.S.) and umbilical vein (U.V.). A negative number indicates a higher value in the U.V.

Day of Gestation	Anim. No.	Female ng/ml	Male ng/ml
100	44W	0.15	1.456
105	24R	1.669	1.310
		-0.642	
110	23W	-14.84	
		-3.84	
115	11W	-4.39	
120	10W	-0.369	0.32
		0	
130	34W	-1.01	1.21
130	33W	1.93	3.23
		1.68	
140	60W	-0.60	0.88
		-0.23	
140	28W	2.10	

(c) The hormonal relationships between the compartments

The relationships between the various compartments changed with advancing gestation and was not the same for all the hormones. The mean ratios for each pregnancy, calculated from individual values, between the compartments for progesterone, oestradiol and oestrone are given in Figure 6-6 and for androstenedione and testosterone in Figure 6-7. Fetal serum levels exceeded maternal levels for all the hormones ($P < 0.01$-0.001, paired t-test) except oestradiol. Fetal serum oestradiol levels were higher than maternal levels only at 140 days of gestation. The relatively highest fetal to maternal ratio was found for testosterone and oestrone.

Amniotic fluid hormone levels were lower or similar to maternal plasma levels prior to 90 days of pregnancy. They remained lower than maternal levels for the remainder of pregnancy for oestradiol and generally for progesterone. ($P < 0.02$-0.05, paired t-test). In contrast, amniotic fluid oestrone, androstenedione and testosterone levels were higher than maternal levels after 100 days ($P < 0.01$-0.001, paired t-test) and the ratio was in the same range as that for the fetal plasma. Progesterone and oestradiol fetal serum levels generally exceeded the anmiotic fluid level ($P < 0.01$, paired t-test) but there was no consistent relationship between fetal serum and amniotic fluid values for the other 3 hormones. Maternal values were not correlated with either the amniotic fluid or fetal serum values. Fetal serum values were not correlated with amniotic fluid values

Fig. 6-6: The progesterone (a), oestradiol (b) and oestrone (c) ratios between the fetal serum and maternal peripheral plasma (●) and between the amniotic fluid and maternal peripheral plasma (o). The mean value for each pregnancy is given. The dashed line (---) gives the 1:1 ratio for reference.

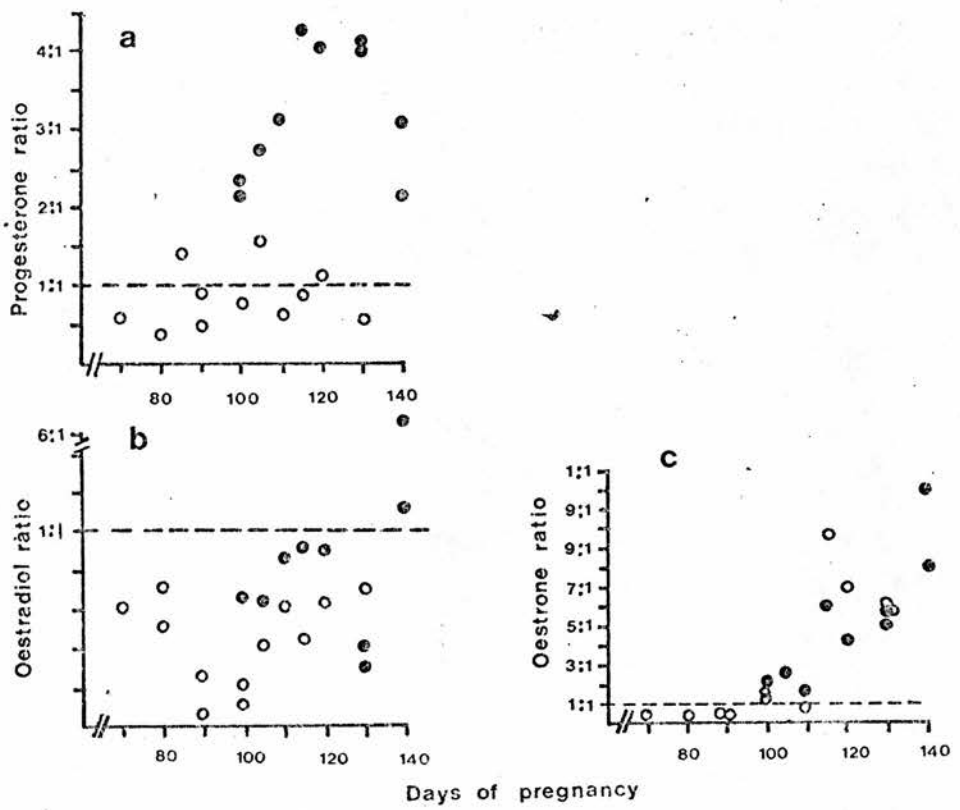
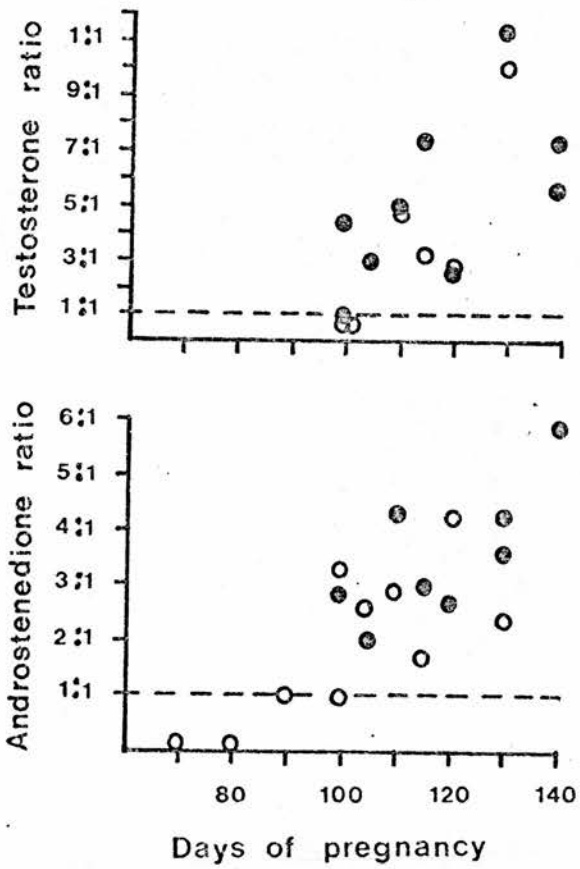


Fig. 6-7: The androstenedione and testosterone ratios between the fetal serum and maternal peripheral plasma (●) and between the amniotic fluid and maternal peripheral plasma (o). The mean value for each pregnancy is given. The dashed line (---) gives the 1:1 ratio for reference.



except for testosterone ($r = 0.839$, $P<0.01$, $n=14$) and oestrone ($r = 0.794$, $P<0.01$, $n=13$).

The mean fetal serum-umbilical vein ratio for each pregnancy is given in Figure 6-8. Progesterone and oestradiol (after day 100) fetal serum levels were always lower than umbilical vein levels but whereas progesterone fetal values became increasingly more similar to umbilical vein values with advancing pregnancy, oestradiol values did not. The difference between the compartments was significant for progesterone ($P<0.01$, $n=18$) and oestradiol ($P<0.01$, $n=18$) (paired t-test). Fetal serum oestrone values were generally lower or similar to umbilical vein values except at day 140 when they greatly exceeded umbilical vein values. In contrast, mean androstenedione values generally exceeded umbilical vein values, the difference being significant ($P<0.02$, paired t-test, $n=16$). The testosterone relationship was more variable and the relationship for testosterone (and progesterone) may have been affected by fetal sex (see sections 6-3bi and v).

(d) The hormonal ratios

The ratios between the hormones were calculated using individual values in all cases and a mean value for each pregnancy was calculated.

(i) Progesterone to oestradiol

The mean progesterone to oestradiol ratio in the maternal, fetal and umbilical vein plasma and the amniotic fluid for each pregnancy is given in Figure 6-9.

Fig. 6-8: The progesterone, P, (○), oestradiol α_2 (○), oestrone, OE_1 (●), testosterone, T (▲) and androstenedione, A (△) ratios between the fetal serum and umbilical vein. The mean value for each pregnancy is given.

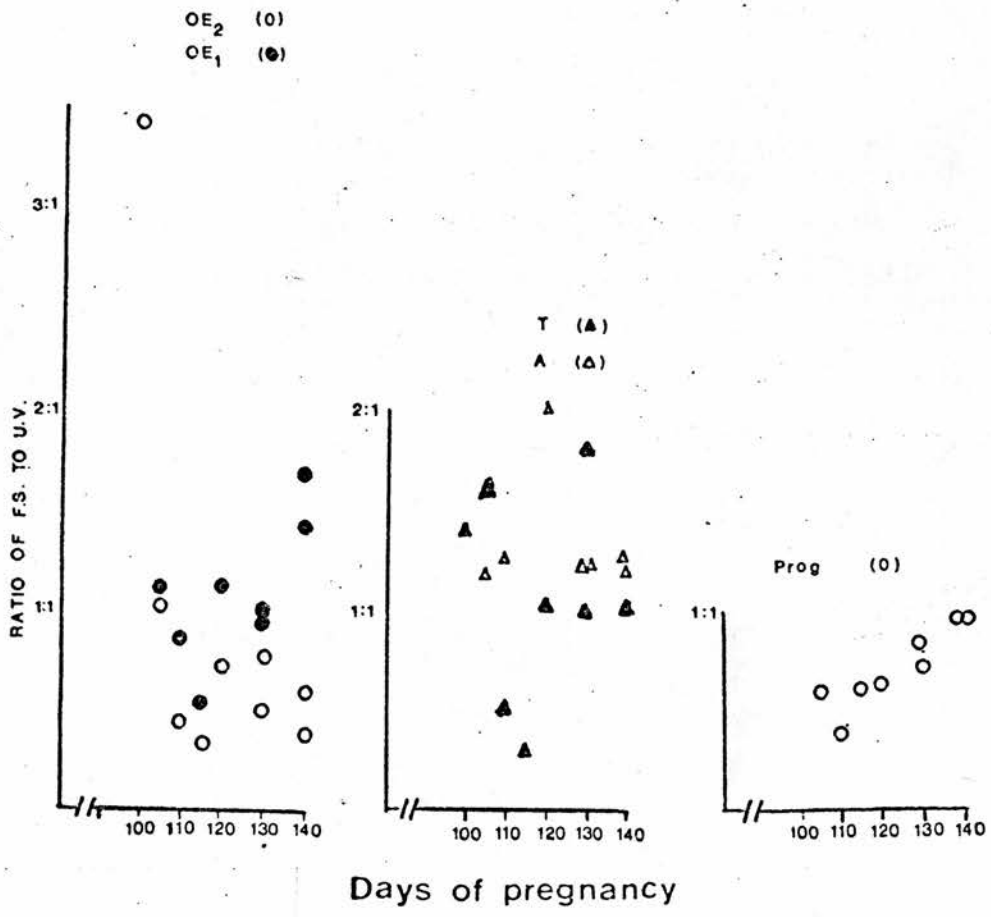
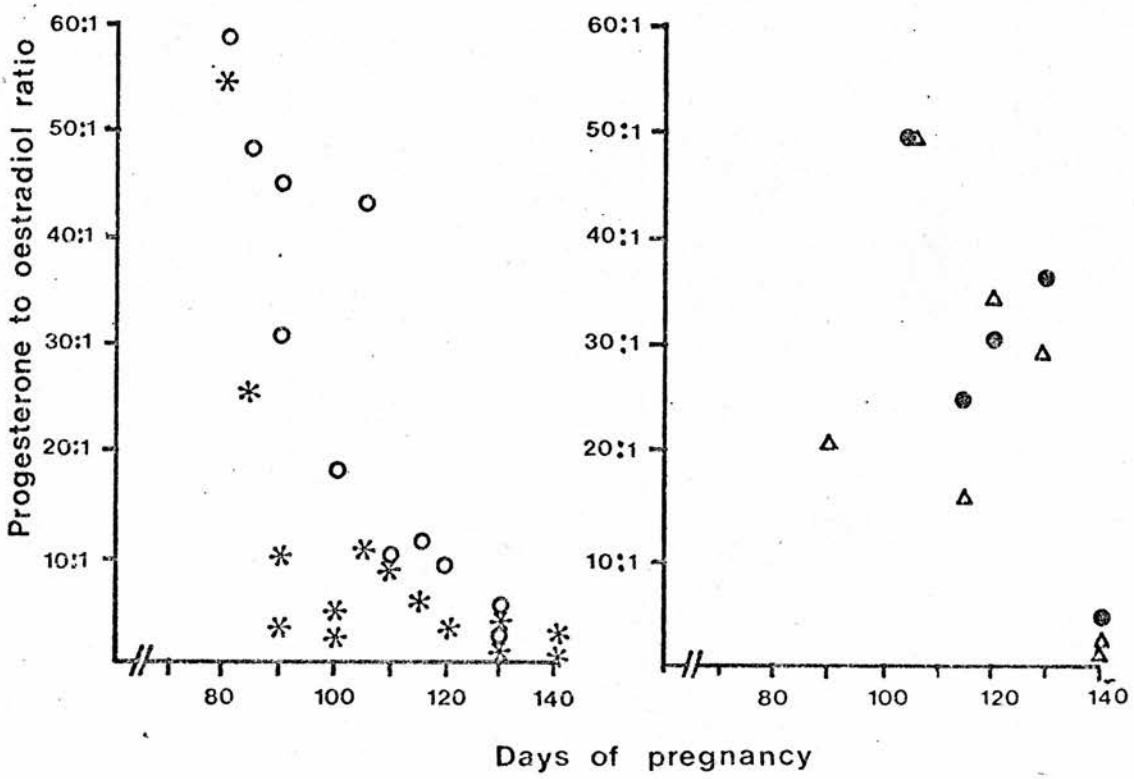


Fig. 6-9: The progesterone to oestradiol ratio in the maternal peripheral plasma (✱), amniotic fluid (o), fetal serum (●) and umbilical vein (Δ). Mean values for each pregnancy are given.



Progesterone values always exceeded oestradiol values. The maternal ratio was generally similar to that reported in Chapter 4. The ratio in the amniotic fluid, fetal and umbilical vein plasma always exceeded the ratio in the maternal plasma although the ratios were similar at 140 days of gestation. The ratio showed a steady decline in the amniotic fluid with advancing gestation whereas no marked decline occurred in the fetal or umbilical vein plasma until 130-140 days of pregnancy. Progesterone and oestradiol were not correlated in the fetal, umbilical vein plasma or in the amniotic fluid.

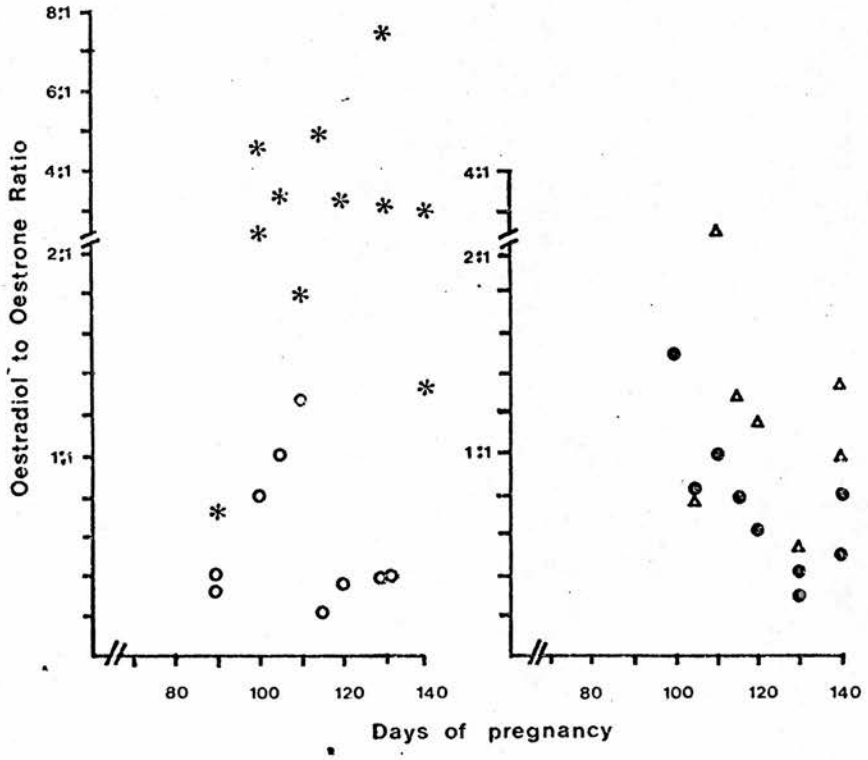
(ii) The oestradiol to oestrone ratio

Figure 6-10 shows the mean oestradiol to oestrone ratio in the maternal, fetal and umbilical vein plasma and in the amniotic fluid for each pregnancy. The maternal ratio was similar to that reported in Chapter 4, with oestradiol predominating over oestrone after 100 days of gestation. With the exception of the pregnancy at 110 days, the ratio was lower in all 3 compartments than in the peripheral plasma and generally oestrone was the major oestrogen between 100 and 110 days. The ratio in the umbilical vein was variable but always exceeded the ratio in the fetal plasma after day 105. Oestrone predominated over oestradiol in the fetal plasma except at 100 days of gestation and the ratio tended to decline with advancing gestation.

(iii) The progesterone to testosterone ratio

The progesterone to testosterone ratio

Fig. 6-10: The oestradiol to oestrone ratio in the maternal peripheral plasma (✱), amniotic fluid (o), fetal serum (●) and umbilical vein (Δ). Mean values for each pregnancy are given.



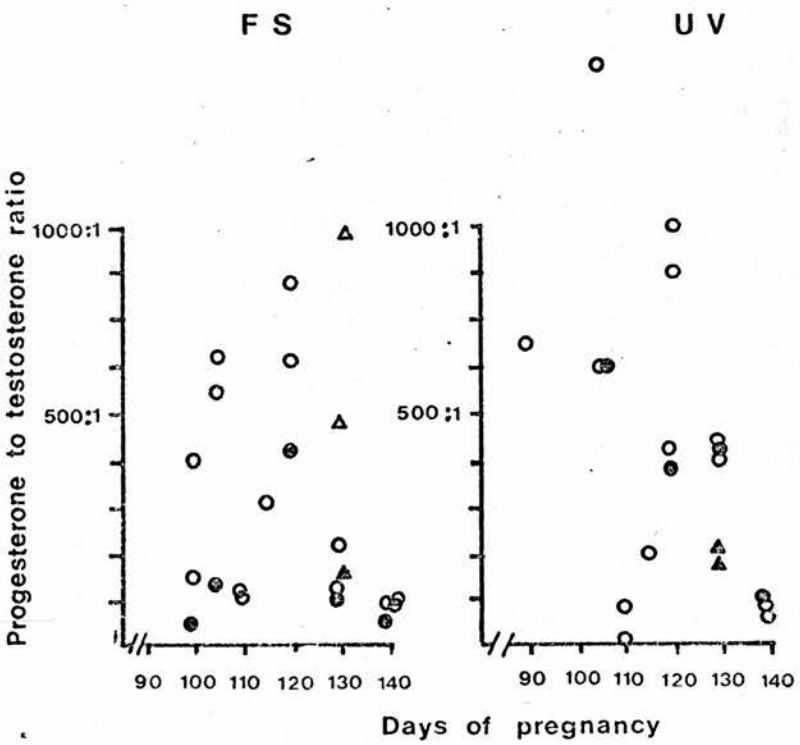
for the fetal plasma and umbilical vein is shown for individual animals in Figure 6-11. The ratio was higher for females than males within a pregnancy ($P<0.01$, $n=6$, paired t-test) and tended to be higher for females than males overall, although there was considerable overlap and the difference was not significant. There was not a comparable sex difference in the umbilical vein.

6.4 Discussion

This is the first study to report on the fetal hormonal environment in the marmoset monkey. Hormonal values ratios and trends were reported for the maternal, umbilical vein and fetal plasma and the amniotic fluid.

Marmoset amniotic and fetal samples were not obtainable until mid-gestation which is considerably later than in the human or rhesus. The relatively slow development of the marmoset fetus during the first half of gestation (Phillips, 1976; Chapter 7) quite likely means that samples have been taken from comparable stages of development. Hampton and Taylor (1971) reported on gonadal development in marmosets and related their data to crown-rump lengths (CRL). Relating their CRL measurements to ours, which were obtained from accurately dated pregnancies, (Chapter 7), allowed the gonadal development to be approximated to a gestational age. The earliest time at which sex could be differentiated was at about day 75 of gestation. This finding was confirmed by O (pers. comm.) utilising gonadal tissue obtained from the fetuses in the

Fig. 6-11: The progesterone to testosterone ratio in the fetal serum , and umbilical vein in female (O,△) and male (●,▲) fetuses. Circles (O,●) and triangles (△,▲) distinguish between fetuses from different pregnancies at the same stage of gestation.



present study. Interstitial cells, the presumed source of testicular steroid hormones, (Niemi, Ikonen and Hervonen, 1967; Pelliniemi and Niemi, 1969), made up a major portion of the testis and appeared glandular after 90 days of gestation. Organ size increased with glandular interstitial cells becoming the major cell type present between 90 and 100 days of gestation. Glandular tissue persisted until birth with a gradual loss occurring shortly thereafter and which was complete by 1 month of age. Amniotic fluid samples were obtained after 70 days and fetal plasma after 90 days, and therefore probably encompassed the period of sexual differentiation.

(a) Progesterone

Progesterone in the fetal plasma followed the same pattern as the maternal peripheral plasma with an increase in levels after 90 days of gestation and a decline during the last week of gestation. The placenta was the most likely source of progesterone in the maternal plasma after 90 days of gestation (see Chapter 5) and in the fetal plasma as umbilical vein levels were significantly higher than fetal levels. A similar finding was made in the human (Hagemas and Kittinger, 1973) and the rhesus (Hagemas and Kittinger, 1972). Infusion of tritiated progesterone in the human (Escarcena, Clark and Gurpide, 1978) and sheep (Tseng, Adamsons, Gaziano, Roberts and Gurpide, 1978) confirmed that the direct secretion of placental progesterone into the umbilical vein accounted for over 90% of the hormone found in the fetus. The

decline in marmoset fetal plasma progesterone before birth was also likely to be related to a lower placental contribution as indicated by lower umbilical vein levels and by the relatively lower placental progesterone content and progesterone secretion *in vitro* that was found at this time (Chapter 5). Changes in blood flow probably would not account entirely for the decline as both oestrogens increased at this time.

An additional source of fetal progesterone during late pregnancy may be the fetal adrenal. Fetal adrenal conversions of pregnenenolone to progesterone have been shown (Solomon and Leung, 1972) and the increase in amniotic fluid progesterone values in late pregnancy in the rhesus was suppressed by betamethasone although maternal progesterone levels were not affected (Challis *et al*, 1977b).

The difference between umbilical vein and fetal plasma indicates a metabolism of progesterone by the marmoset fetus. Human and rhesus fetal tissues, particularly the liver and adrenals, extensively metabolise progesterone (human: Bird, Wiqvist, Diczfalusy and Solomon, 1966; Solomon, Bird, Ling, Iwamiya, and Young, 1967; rhesus: Leung and Solomon, 1972). The products formed in these 2 species varies and it is not known to what degree the marmoset would resemble either species. In addition, the fetal testis metabolises progesterone. A major *in vitro* progesterone metabolite in fetal (Coffey and Johnsonbaugh 1979) immature (Mizutani, Tsujimura, Akashi and Matsumoto, 1977) and adult (Hoschoian and Brownie, 1967) rhesus

monkey testis is 17-hydroxy-4-pregnene-3,20,dione. This steroid is also the chief *in vitro* progesterone metabolite in adult marmoset testis (Preslock and Steinberger, 1977b).

There was not an overall sex difference in marmoset fetal progesterone, but comparison between fetuses within the same pregnancy showed higher fetal plasma levels associated with a female than a male fetus. The lack of an overall sex difference in levels may partly be due to gestational hormonal changes which were reflected in both fetuses. There was no corresponding sex differnce in umbilical vein progesterone levels either overall or within a pregnancy. In view of the shared placental circulation and/or the attachment of both male and female fetuses to one placental disc (Chapter 7), it is perhaps not surprising there is no sex difference in umbilical vein plasma in male-female pregnancies.

Higher progesterone levels were also found in the female fetal rhesus than in the male, but unlike the marmoset, these higher female levels were also associated with higher umbilical vein progesterone levels (Hagemenas and Kittinger, 1972), a greater metabolism of progesterone by the female fetus (Hagemenas and Kittinger, 1972; MacDonald, et al, 1973), and a greater in vitro secretion of progesterone by placental tissue from a pregnancy with a female fetus than with a male (Hagemenas and Kittinger, 1974). Thus,

a sex influence on placental progesterone biosynthesis or metabolism, and on fetal metabolism was indicated for the rhesus monkey. Results from studies in the human are equivocal: a somewhat similar finding to the rhesus was made by Hagemenas and Kittinger, (1973), but not by others (Tulchinsky and Okado, 1975; Effer et al, 1973 ; Dawood and Helmkamp, 1977).

Since the data in the present study did not show a sex difference in the amount of progesterone in the umbilical vein, or a higher placental secretion of progesterone in vitro from placental tissue taken from pregnancies with only female fetuses (days 100,110,115,140, Figure 5-24), it seems likely that the higher female fetal progesterone levels may be due to a lower, rather than a higher progesterone metabolism by female fetuses ; although other factors may play a role. An ovarian progesterone source is an obvious candidate, and there are repo rts of fetal ovarian progesterone synthesis (Rice, Jacks, Smith and Sternberg,1971; Wilson and Jawad, 1979). However, marmoset fetal ovaries taken after 80 days of gestation and maintained in organ culture, did not secrete measurable amounts of progesterone (unpubl'd data), and the general conclusion of most workers, is that most, if not all, of the circulationg progesterone in the fetus is derived from the placenta (Diczfalusy, 1974). Whether there is a sex difference in the progesterone-binding protein, transcortin, which is a likely mechanism for maintaining the higher fetal than maternal progesterone levels (Klopper and Fuchs, 1977) is not known.

Progesterone values in the fetal plasma and in the amniotic fluid followed the general trend as the maternal

plasma although fetal plasma values were severalfold higher than those in the maternal peripheral plasma and amniotic fluid values were generally lower than those in the maternal plasma. Fetal plasma values also exceeded maternal plasma values in the human, baboon and rhesus, but in the rhesus, fetal plasma values declined as pregnancy progressed (human: Hagemenas and Kittinger, 1973; baboon: Dawood and Fuchs, 1980; rhesus: Thau , Lanman and Brinson, 1976). Some species, e.g. the rat, show higher maternal than fetal progesterone values (Weisz and Ward, 1980) and this may reflect differences between species in the relative ovarian-placental importance to progesterone levels and/or differences in the fetal and maternal level of progesterone binding protein.

Comparison of the progesterone pattern and levels between the amniotic fluid and the fetal and maternal compartment reveals considerable variation among the primate species. In the human, amniotic fluid progesterone values declined with advancing gestation and were significantly lower than maternal levels after mid-gestation. (Johansson and Jonasson, 1971; Warne et al, 1977; Nagamani, McDonough, Ellegood and Mahesh , 1979). In the rhesus, the amniotic fluid levels were also significantly lower than maternal values but showed an increase that was greater than the corresponding change in the maternal peripheral plasma (Challis, et al, 1977b). In the marmoset amniotic fluid values were also generally lower than maternal values but were severalfold higher than the human at the end of gestation although peripheral values were

similar. This is because marmoset amniotic fluid values increased after 90 days of pregnancy and remained high.

The reason for these differences between the species is not known. Common to all these species though is a lack of correlation between amniotic fluid values and maternal or fetal values. This may be related to the route of entry of hormones into the amniotic fluid, which has not been firmly established. The different patterns in the amniotic fluid between the hormones may relate to different routes of entry and the hormone's solubility and degree of conjugation. In addition, the factors involved probably change with advancing gestation (Behrman, Parer and de Lannoy, 1967; Klopper, 1970; Dawood, 1977).

(b) Oestrogens

Concentrations of the 2 oestrogens, oestradiol and oestrone, in marmoset fetal plasma were not related to their concentrations in the maternal peripheral vein. In addition, whereas fetal oestradiol levels were lower than maternal peripheral levels between 100 and 130 days of gestation, oestrone levels generally greatly exceeded those in the maternal peripheral plasma. This resulted in a shift in the oestradiol to oestrone ratio between the maternal and fetal compartments in favour of oestrone; and in the majority of cases the ratio was reversed between the 2 compartments. Similar findings have also been reported for the human (Tulchinsky, 1973; Shutt, Smith and Shearman, 1974; Reyes, Boroditsky, Winter and Faiman, 1974)

and rhesus (Resko, 1974b; Resko, Ploem and Stadelman, 1975; Novy, 1977), despite the large interspecies differences in oestrogen concentrations.

The placenta may be the major source of fetal oestrogens and be partly repsonsible for the reversal of the oestrogen ratio in the fetal plasma. Umbilical vein oestrogen values were generally higher than the fetal plasma values and compared to the utero-ovarian vein and maternal plasma, the oestradiol to oestrone ratio shifted in favour of oestrone in the umbilical vein. Larger quantities of oestrogen in the umbilical vein than umbilical artery, containing greater amounts of oestrone than oestradiol was also found in the human (Tulchinsky, 1973; Kenny et al, 1973; Shutt et al, 1974) and rhesus (Resko et al, 1975). In vivo and in vitro perfusion studies in the human also showed a greater secretion of oestrone than oestradiol from the placenta to the fetus (Bolte et al, 1964a,d). In the rhesus also, there may be an adjustment in favour of oestrone production by placental tissue during late pregnancy (Dierschke, Wehrenberg, Wolf, Clark and Robinson, 1978). The shift in ratio between the umbilical vein and the utero-ovarian vein may also be indicative of a selective secretion of oestradiol or oestrone either to the maternal or fetal compartment. A preferential secretion was found in the rhesus monkey; whereas oestradiol was selectively secreted to the maternal compartment, oestrone was not (McCarthy et al, 1980). Although the marmoset placenta contained more oestradiol

than oestrone in late gestation, the ratio was lower in the placental tissue than in the peripheral plasma (Chapter 5) but higher than in the umbilical vein, which may suggest that an explanation similar to the rhesus may be applicable to the marmoset.

Other factors may also be involved in the differences in ratio between the 2 compartments. In the marmoset the difference between the umbilical vein and fetal plasma concentrations was greater for oestradiol than for oestrone and the oestradiol:oestrone ratio was lower in the fetal plasma than the umbilical vein. Oestradiol may be more rapidly metabolised or conjugated than oestrone, in the fetal compartment than the maternal. Differences between the 2 hormones in their metabolic clearance rates between the 2 compartments was shown for the rhesus (McCarthy et al, 1980). Higher levels of oestrogen binding protein, which has a greater affinity for oestradiol than oestrone (Westphal, 1971), in the maternal than in the fetal plasma may also aid in maintaining higher maternal oestradiol values.

Interconversions of oestrone and oestradiol may account for the difference between the oestradiol: oestrone ratio in the umbilical vein and fetal serum. Fetal ovarian or adrenal secretion were also suggested to be of possible significance in the rhesus (Resko et al, 1975; Resko, 1977). These factors are probably more negligable in the marmoset due to the comparatively greater amounts of oestrogen secreted by the placenta to the fetus.

Also, oestrone and oestradiol were not correlated in either the fetal or maternal plasma in the marmoset, whereas they were correlated in the rhesus. No oestrogen was measurable in marmoset fetal ovarian cultures (unpublished observation).

Marmoset maternal peripheral oestrogen levels, particularly oestradiol, were highest during the last week of gestation (Chapter 4). Significant changes also occurred in the fetal compartment. Fetal plasma oestrone values greatly exceed umbilical vein values and there was an increase in the umbilical vein and fetal plasma oestrogen values between 130 and 140 days which was of much greater magnitude than that in the peripheral plasma. This resulted in both oestrogens exceeding maternal peripheral plasma levels and a larger decline in the progesterone to oestradiol ratio in the fetal than in the peripheral plasma. Human (Shutt et al, 1974), rhesus (Resko et al, 1975) and sheep (Challis, 1971; Strott, Sundel and Stahlman, 1974) fetal oestrogens also increased at the end of pregnancy. The increase appeared to be associated with maturation of the fetal pituitary-adrenal axis (Bedford et al, 1972) and the rise of corticosteroids which was related to the onset of parturition in the sheep (Challis, Kendall, Robinson and Thorburn, 1977) although not necessarily in the primate (Novy, Walsh and Kittinger, 1977; Novy, 1977). There was no corresponding increase in marmoset fetal androgens during the last week of gestation when oestrogen levels increased. It was suggested that the oestrogen increase

in the sheep resulted from the effects of fetal cortisol on placental enzymes rather than an increase in production of androgen steroids (Challis et al, 1977; Challis, 1980). The cause and significance of the changes in fetal oestrogen values in late pregnancy, the functioning of the marmoset pituitary-adrenal axis and any role of the fetus in the timing of parturition must await further studies in the marmoset.

(c) Androgens

Testosterone and androstenedione values in the fetal serum and amniotic fluid did not follow the same pattern as the maternal peripheral plasma. This probably reflects to an extent the different sources of androgens in the maternal and fetal compartment. In addition to the placenta, the maternal and fetal gonads and adrenals contribute substantially to androgen levels in other species (reviews: Diczfalusy, 1974; Gandy, 1977; Resko, 1977; Winter et al, 1977).

Although detailed information is not available on marmoset gonadal development, the increase in fetal testosterone levels after 90 days of gestation probably correlated with the appearance of glandular interstitial cells (Hampton and Taylor, 1971). Testosterone levels remained high until birth and interstitial cell development persisted until birth (Hampton and Taylor, 1971). Prior to 90 days of gestation, it may be inferred that fetal testosterone levels would also be low as fetal plasma and amniotic fluid levels were low at 90 days and amniotic fluid

levels were low prior to 90 days. Amniotic fluid testosterone levels reflected fetal serum levels during later gestation in the marmoset and they have an unequivocal sex difference and reflect circulating levels reasonably well in the human (Dorner, Stahl and Baumgarten, 1972; Giles, Lox, Heine and Christian, 1974; Dawood and Saxena, 1977; Warne et al, 1977). It is possible that a relatively short lived rise in testosterone, which in the rat results in distinctive male levels for only 2 days (Weisz and Ward, 1980), was missed in the present study. Other primates have a longer period of sexual differential and divergent hormonal levels during fetal life.

However, despite the possible correlation between testosterone levels and gonadal development, the increase in fetal and amniotic fluid testosterone levels that occurs after 90 days of gestation may not be primarily due to a gonadal secretion but to the increased placental secretion of androgens to the fetus. This was indicated by the increased umbilical vein values, the lack of a sex difference and that fetal values did not necessarily exceed umbilical vein values. The high testosterone level secreted by the placenta was unlikely to be due primarily to androgen secretion by the male gonad that had not been aromatised by the placenta to oestrone (Bolte et al 1964a-d) inasmuch as the highest umbilical vein (and fetal) testosterone levels were found in a pregnancy with only female fetuses. Also, in 2 other pregnancies with only female fetuses, testosterone levels were similar to

those with male fetuses.

This contrasts with the situation found in man and rhesus monkey. Although the placenta secreted androstenedione and testosterone to the fetus (Lamb, Mancuso, Dell 'Acqua, Wiqvist and Diczfalusy, 1967), the bulk of the fetal testosterone in the male is from the fetal gonad. This results in a significant sex difference in testosterone levels at least during the period of sexual differentiation (see reviews) which is abolished by fetal castration (Resko, Malley, Begley and Hess, 1973). This difference either disappears in late gestation or is of small magnitude (Resko, 1970; Abramovich, 1974; Forest, Cathiard and Bertrand, 1973; Diez D'Aux and Murphy, 1974; Reyes et al, 1974; Dawood and Saxena, 1977).

The relatively small amount of testosterone in the female fetal rhesus was attributed to other sources, such as the placenta and peripheral conversions of androstenedione (Resko, 1977). Androstenedione-testosterone interconversions have been reported for the human (Horton and Tait, 1966; Blaquier et al, 1967). In addition, the rhesus fetal ovary and adrenal probably contributed to androstenedione levels (Solomon et al, 1967; Resko, 1977).

These factors may also have a role in the fetal marmoset. Relatively high fetal androstenedione values were found and a conversion factor similar to the human could account for all of the circulating testosterone.

There may be increased fetal adrenal androgen

secretion, particularly androstenedione, at 90-100 days of pregnancy. Marmoset fetal adrenal cultures secreted androstenedione after 90 days of gestation (Chapter 5). Also, androgen levels increased only in the fetal compartment but not in the maternal compartment; and androstenedione fetal serum values were generally higher than umbilical vein values.

Fetal androgens, of adrenal origin, are known precursors for placental aromatisation to oestrogens (reviews: Beling, 1977; Levitz and Young, 1977; and others). Although there was no overall correlation between androgen and oestrogen values, the animals with apparently high androgen values also had unusally high oestrogen values. Also, androgen which was not aromatised to oestrogen in the placenta may be secreted to the fetus (Bolte et al, 1964a-d).

A small source of testosterone in the fetal male that was not in the fetal female, presumably the fetal testis, was however suggested in the present study. Although there was no overall sex difference, testosterone levels were higher in male fetuses compared to their female counterpart in pregnancies in which both sexes were present. Males also had higher fetal plasma testosterone values than umbilical vein values, whereas females more often had similar or lower values in the fetal plasma than in the umbilical vein. However, some females did show differences similar to those in the male and more data would be necessary to confirm these results. Marmoset testis also secreted large quantities of testosterone in vitro after 90

days of gestation, whereas no testosterone was measureable in fetal ovarian cultures (unpublished observations). Although appreciable quantities of testosterone were secreted *in vitro* at 90 days of gestation, amniotic fluid and fetal serum levels were low. Gonads were cultured in the absence of exogenous hormones, and these hormones may alter gonadal production and secretion of androgen (Matsumato, Mahajan and Samuels, 1974; Kalla, Nisula, Menard and Loriaux, 1980). Also, there may be testosterone contained within the gonad prior to its detection in the fetal serum as in the human (Reyes, Winter and Faiman, 1973; Winter *et al*, 1977). Thus it seems likely that although there probably is a fetal testicular component to testosterone levels, it is low compared to other sources in the fetus. Any secretion earlier in gestation was insufficient to be reflected in the amniotic fluid, which is unlike the human and rhesus (see reviews); species in which all other hormonal levels tended to be lower than those in the marmoset. In fact, although fetal testosterone levels may appear relatively high compared to those in the human and rhesus, they are relatively less elevated than are the fetal oestrogen and progesterone values compared to these species. A comparison of the amniotic fluid values illustrates this point (Table 6-9).

The increase in androgen levels in the fetus coincided with the increased levels of other steroid hormones. This temporal relationship may be important. As seen in Table 6-9, relative to other hormonal levels, the

Table 6-3. Comparison of the hormonal concentrations in the amnniotic fluid for the human, rhesus and marmoset. Data was obtained from Warne et al, 1977; Dawood, 1977; Nagamani et al, 1977 for the human, Challis et al, 1977a for the rhesus and the present study for the marmoset. P4 = progesterone, OE_2 = oestradiol, OE_1 = oestrone, A = androstenedione, T = testosterone.

	P4	OE_2	OE_1	A	T
Human	5-69	1.2-3.1	2.5-4.4	0.01-1.02	0.01-0.73
Rhesus	0.5-1.4	0.05-0.2	0.05-1.2	0.1-1.3	
Marmoset	32-650	0.7-34	1-89	0.8-45	0.2-1.5

fetal marmoset is not exposed to particularly high testosterone levels. However, if fetal testosterone levels increased prior to day 90, the marmoset fetus would be exposed to comparatively high levels. A coincident elevation in progesterone and oestradiol may be important as antagonistic actions of these 2 hormones have been shown in several instances (reviewed: Resko,1974; 1977). Resko also suggested that the ratio of androgen to antiandrogen may be important in sexual differentiation and there was a significant sex difference in the testosterone to progesterone ratio in the rhesus. Although there was some overlap overall, there was a significant sex difference in this ratio in the marmoset in pregnancies with both male and female fetuses. The coincident rise in oestrogen may be important also as there may be an oestrogen-mediated rise in binding proteins which may inactivate much of the circulating testosterone(Pearlmen et al,1967)

Since testosterone has been implicated for certain aspects of fetal sexual differentiation in other primates (reviews;Sect.6.1), the similarity between male and female fetuses in androgen profiles suggest that either testosterone does not have a comparable role in the marmoset, or that other factors are relevant. These factors may include, in addition to the hormonal ones discussed above, that there is a local effect on sexual differentiation prior to day 90, when circulating levels appear to be low. Females may differ from males in their critical period for sexual differentiation and it may occur prior to the increase in circulating testosterone levels. Females may differ in their threshold of sensitivity to androgen and/or ability to convert it to

its more active metabolites. The placenta may also serve a protective function by converting androgen to oestrogen (Ryan et al, 1961). Certainly androgen levels are comparatively low whereas oestrogen levels are comparatively high.

Although there is pre-natal anatomical sexual differentiation, the marmoset may also undergo a post-natal period of sexual differentiation (Abbott, 1979) The relative role of pre- and post-natal behavioural sexual differentiation was not ascertained and there have not been comparable studies in other primates to assess whether the marmoset is unusual among primates in this respect. The marmoset may be unique in its hormonal method of imprinting sexual differentiation because of its unusual embryology. Dizygotic twinning is very common and yet despite the early development of a shared placental circulation and haemtopoietic chimesism, females born cotwin to a male are totally unaffected (See Chapters 5-5 and 6-1 for references). This study has further shown that male and female fetal marmosets develop normally in a similar androgen environment. Whether marmosets can be masculinized in vivo by androgen administration during critical periods of development such as has been done in other species, is not known (human : Wilkins, Bongiovanni; Clayton, Grumbach and Van Wyk, 1955; Wilkins, 1960; rhesus: VanWagenen and Hamilton, 1943; Goy, Wolf and Eisele, 1977; sheep: Short, 1974; Clarke, Scaramuzzi and Short, 1977).

Phillips (1969) found no effects when androgen

was administered to pregnant females, but the dosages used were low and infrequent and the stages of gestation were not given. (See also Chap. 8).

(d) LH/CG

CG is widely implicated as having a role in stimulating fetal testicular testosteone secretion, and its pattern during pregnancy has been correlated with sexual differentiation (see reviews Section 6-1). The earliest sexual differentiation in the marmoset probably occurs around 70-80 days of gestation when maternal peripheral LH/CG levels in the maternal plasma are still high but beginning to decline. Amniotic fluid LH/CG levels were not measurable at this time, which is perhaps not surprising in view of the large concentration gradient found in the human between the maternal and fetal compartment (Reyes et al, 1974; Geiger, Kaiser and Franchimont, 1971). But unlike the human and rhesus, maternal LH/CG had declined to low levels and amniotic and fetal LH/CG levels were unmeasurable when interstitial cells and testosterone appear between 90-100 days of gestation. Gonadal development and testosterone secretion possibly occurs later in gestation relative to maternal CG values than is found in the human. In the human, levels of hCG are high enough throughout gestation in the fetal circulation to maintain testicular steroidogenesis (Clements, Reyes, Winter and Faiman, 1976; Kaplan and Grumbach, 1978), whereas in the rhesus, at least in the latter part of gestation, a more likely gonadotrophic stimulus was

suggested to be the fetal pituitary (Hodgen, Niemann and Tullner, 1975). The assay used in the present study may not be sensitive enough to measure low levels of marmoset fetal LH/CG and to elucidate the gonadotrophic control of gonadal development in the marmoset, it would be desirable to have assays that fully distinguish between marmoset LH and CG. It would also be necessary to confirm gonadal hormone secretion in relation to the stage of gestation, to gonadotrophin stimulation, and to gonadal development. The gonadal tissue obtained from the timed pregnancies in this study have yet to be fully analysed.

6.5 Chapter summary

(1) All hormonal values in the amniotic fluid and umbilical vein increased at 90 to 100 days of pregnancy coincident with the increased placental content of all these hormones and with the increased maternal peripheral progesterone and oestradiol levels. Fetal serum hormonal values were also elevated at day 100, and it was suggested that a coincident increase in hormonal levels may be physiologically significant.

(2) Fetal serum values and the hormonal ratios were dissimilar to those in the maternal peripheral plasma. All fetal serum values, except oestradiol, were higher than those in the maternal peripheral vein. Like other primate species, the fetal oestradiol to oestrone ratio shifted in favour of oestrone.

(3) Hormonal values in the amniotic fluid changed independently of each other and except for testosterone and oestrone, were not correlated with the fetal serum values. None of the amniotic fluid hormonal values correlated with the maternal peripheral plasma.

(4) Fetal utilisation and production of each hormone probably varied as indicated by variations in the fetal serum-umbilical vein relationship.

(5) Overall, male and female fetal marmosets develop in a similar hormonal environment and any gonadal contribution to male testosterone levels was probably small compared to the placental contribution. There was a sex difference for progesterone and testosterone, and for the progesterone to testosterone ratio between male and female fetuses within a pregnancy.

CHAPTER 7 : PLACENTAL AND PRENATAL DEVELOPMENT

7.1 Introduction

Studies on placental and prenatal development have been confined mainly to man, the rhesus monkey and baboon (reviews: Tanner, 1978; Hendrickx, 1972, 1971; Schultz, 1956, 1969; and others). Early studies in the Callitrichidae species utilised specimens mainly caught in the wild, (Wislocki, 1929, 1932, 1939; Hill, 1926, 1932; Hill and Hill, 1927) and the data from later studies was not generally associated with a precise gestational age (Phillips, 1976; Hampton, 1975). These studies suggested that the embryology of the marmoset was unusual and that the timetable of embryonic development may be different from other primate species.

Does the marmoset have a distinct prenatal growth pattern from other primates or does it fit in with some of the generalised concepts of prenatal development? How does the prenatal development timetable relate to the hormonal changes occurring during pregnancy? Can a set of growth standards be established for utilisation by other investigators? In order to answer these questions, a systematic study of prenatal development was undertaken in conjunction with the hormonal studies already reported.

7.2 Procedures

Samples were obtained following hysterotomy (Chapter 2.6). The sampling regime was given in Chapter 2.7.

(a) Placental measurements

There were two placental discs in every pregnancy. Each disc was weighed and measured separately. The major (a) and minor (b) axes were measured and the placental area was estimated using the formula for an oblate spheroid, $A = \pi \frac{ab}{4}$.

(b) Embryonic and fetal measurements

All measurements were taken 2-3 hours following removal of the feto-placental unit. Measurements were taken prior to fixation in specimens after 80 days of pregnancy. Embryos before day 70 of pregnancy were fixed in 10% buffered formalin just prior to measurement.

The dimensions of the yolk sac (length x width) were taken between 30 and 100 days. The length, width and thickness of the 30 day embryo was taken from post-fixation sections. The greatest length (day 40) or crown-rump length of 50-80 day embryos was taken in their natural position using a grid under a dissecting microscope (Wild M5 stereo dissecting microscope). Crown-rump length of fetuses more than 90 days old was taken with calipers. 19 other body dimensions were taken and some measurements were calculated from these basic measurements (Table 7-1). A diagramatic representation of those measured (based on the human fetus) is shown in Figure 7-1. These skeletal measurements were possible only after 90 days of pregnancy. The definition of these measurements is given in full detail by Schultz (1929) and the techniques used were similar.

Table 7-1. Body measurements (taken according to the technique of Schultz, 1929) and calculated measurements. The numbers refer to the diagramatic representation in Figure 7-1

TRUNK

1. Crown-rump (C.R.L.)
2. Trunk height (T.H.)
3. Shoulder breadth (S.B.)
4. Hip breadth (H.B.)
5. Chest breadth (C.B.)

   Chest depth (C.D.)

   Chest circumference (C.C.)

LIMB

6. Thigh length (T.L.)
7. Knee heel (K.H.)
8. Leg length (L.L.)
9. Foot length (F.L.)
10. Upper arm length (U.A.L.)
11. Forearm length (F.A.L.)
12. Hand length (H.L.)

HEAD

13. Head width (H.W.)

    Head length (H.L.)
14. Head height (H.H.)
15. Total face height (T.F.H.)
16. Upper face height (U.F.H.)

Tail length

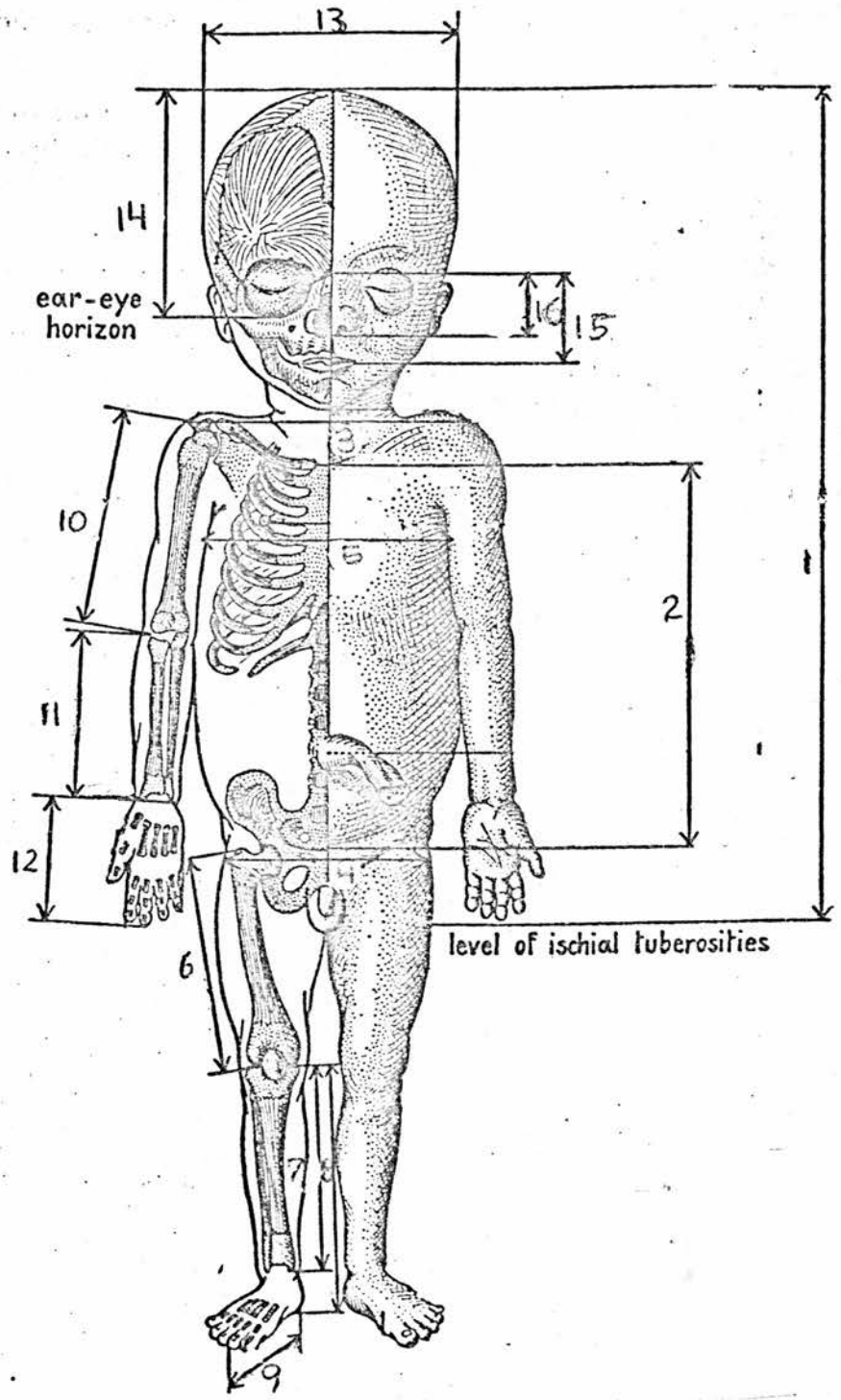
CALCULATED MEASUREMENTS:

(U.L.L.) Upper limb length = U.A.L. + F.A.L. + H.L.

(L.L.L.) Lower limb length = T.L. + K.H.

(A.H.D.) Average head diameter = (H.W. + H.L. + H.H.) ÷ 3

Fig. 7-1: Diagrammatic representation of measurements. Human fetus with skeletal parts shown on the right half of body. The numbers refer to the particular measurement. (See Table 7-1.) (taken from Schultz, 1929)



The accuracy of the measurements was assessed in 2 instances by triplicate determinations (taken on the same day). The coefficient of variation was less than 6% in all cases.

All body and organ weights were taken on an Oertling balance which weighed to the fourth decimal place. The body weight was taken after 70 days of gestation and organ weights after 80-90 days.

Most results are presented as mean values for each pregnancy as there was little difference between fetuses within one pregnancy and there was no apparent sex difference in any of the measurements. The timed pregnancy at 100 days consisted of triplets, one fetus of which was abnormal in appearance and size. Mean values for the 2 normal fetuses have been given and where applicable, individual values from the abnormal fetus.

(c) Birth and adult measurements

Body weight and dimensions were taken on 4 sets each of normal triplets and twins on either the day of birth or on day 1. Newborn organ weights were taken from still births or from animals dying within 1 day of birth.

Adult body weight and dimensions were taken in 6 males and 6 females more than 500 days of age. The animals were anaesthetized with 0.4ml of Saffan prior to measurement.

Adult organ weights were taken from autopsy records of 7 males and 7 females. (S.F. Lunn, pers'l commun.) Animals with obvious organ abnormalities or infections were

excluded. The body weight of these animals was lower than the mean colony body weight.

The singleton, twin and triplet birth weight and the adult body weights used in this paper are the mean from 20, 226, 122 and 64 animals respectively. (S. F. Lunn, pers comm)

7.3 Results

(a) External description

The data for the individual embryos from timed pregnancies between 30 and 80 days are given in Table 7-2. There was a distinct growth in the crown-rump length (CRL) during the 10 day intervals but individuals within a pregnancy or from the same time in gestation had similar measurements. The yolk sac was prominent after the 50th day and continued to increase in size until the 80th day. By 110 days, only vestigal amounts remained. All embryos were enclosed by their own amniotic membrane in a common exo-coelom enclosed by a common chorion.

The axis of the embryonic disc was defined in the 30 day embryo by the primitive streak. The embryo measured approximately 0.06 x 0.05 x 0.02cm. In the 40 day embryo, the neural folds were forming over the cranial end and the body stalk was prominent at the caudal end. The greatest length of the embryo was 0.10m.

By 50 days of gestation, there was a marked cranial and dorsal flexure. 7 to 9 somites and the neural tube had formed. The optic primordia formed bulges

Table 7-2. Embryonic crown-rump length (CRL) (cm), and weight (grams); the number of somites visible, the dimensions of the yolk sac (length times width = LXW), the chorionic diameter (cm) and the placental weight (g) for timed pregnancies between 30 and 80 days of gestation. All of the pregnancies consisted of twins.

Day of Pregnancy	CRL (cm)	No Somites	Weight (gm)	Yolk sac (LXW)	Chorionic diameter (cm)	Placental weight (g)
30					0.45	0.0313
40					0.75	0.2046
40					0.75	0.2082
50	0.24 0.21	8 9		0.6x0.4	1.50	0.8317
50	0.22	7		0.5x0.4	1.35	0.7984
60	0.58 0.60	25		0.5x0.4	1.85	1.6065
60	0.60 0.61	~ 28			1.80	1.2512
70	1.10 1.00		0.1677 0.1044	0.8x0.6 0.8x0.5	1.90	1.3475
80	2.16 2.05		0.8076 0.7611	0.8x0.8 1.2x0.8	2.95	4.8795
80	2.26 2.15		0.8383 0.7350	0.8x0.7 0.9x0.8	2.80	4.8572

in the cranial region and the heart formed a bulge on the ventral surface. The yolk sac was nearly 3 times the embryo length.

At 60 days of gestation, there were 3 visceral arches separated by 3 visceral grooves. The optic vesicles were on the lateral side of the head and appeared as opaque circles with transparent centres. The otic vesicle opened on the surface adjacent to the 1st visceral groove. The oral fossa were large and opened wide. Somites were visible from below the otic pit. The cervical sinus was present in the caudal arch region. The heart was prominent. There were forelimb buds but no hind limb buds. The tail was rounded, blunt, untapered and pointed cranially.

By 70 days of gestation, the forelimbs had differentiated into hand and arm regions and were starting to curve over the body. The hind limbs were beginning to show division into foot and leg-thigh regions and were pointed craniomedially. There were no digital rays. The cervical flexure was approximately 30^{o}. The heart was comparatively large. The mandibular and hyoid arches were prominent. There was no development of the external acoustic meatus. Retinal pigmentation was beginning.

At 80 days of gestation, there was no cervical flexure. The limbs had lengthened with the hands overlapped and palms pointed caudally. The interdigital tissue between the fingers and toes had decreased. The eyelids had developed and covered approximately two-thirds

of the eye. The tragus and antitragus were evident in the auricle. The tail was pointed.

The external features had more fully developed by 90 days of gestation. The ear had developed, the nasal palate had closed, the nose and mouth were separated. By 100 days of gestation, hair follicles were beginning to appear, particularly around the face. Nails and teeth had developed. By 110 days of pregnancy hair follicles were evident over the whole body. Fur growth had started by 120 days of gestation. By 130 days of pregnancy, the fetus resembled a newborn marmoset.

The attachment of the fetus to the placental disc varied from marginal to central. In some instances the umbilical veins divided before reaching the placenta. The umbilical veins from the fetus attached to one or both of the placental discs or even to the placental anastomoses. A placental disc may have had 0 to 3 fetuses attached to it.

(b) Placental growth

There were 2 placental discs present in every pregnancy which were connected by prominent blood vessels. The attachment of the placental discs to the uterine wall was dorsal and vential.

There was no obvious primary and secondary placental disc as the 2 discs were similar in size and weight. The mean percentage of the size and weight of the smaller disc to the larger disc was 93.4 ± 6 (s.d. n = 25) and 93.0 ± 5 (s.d., N = 25) respectively. In

pregnancies in which there were a different number of fetuses attached to each placental disc, the 2 placental discs did not show any greater difference in size or weight (range 88-98%) than those with an equal number of fetal attachments (range 88-100%).

The total placental weight and area from all pregnancies between 30 and 140 days of pregnancy is shown in Figure 7-2. The placenta was approximately 25% heavier and 40% larger from a triplet than a twin pregnancy. The placenta (2 discs) obtained from a singleton pregnancy was lighter and smaller than from a twin pregnancy.

The increase in placental weight (Figure 7-2a) followed a sigmoid curve. The maximum increments in weight gain (1-1.5g /10 days) were found between 60 and 90 days of gestation and thereafter declined to 0.15gm/10 day between 130 and 140 days of gestation. The rate of weight gain was maximal at the earliest period in gestation, declining from 757g /g /10 days between days 30 and 40 to 0.023g /g /10 days between days 130 and 140.

The area growth (Figure 7-2b) was greatest and nearly linear ($10cm^2$/10 day) between 40 and 90 days of gestation. It thereafter declined to $4cm^2$/10 days between days 130 and 140. The rate of area growth was greatest at the earliest period of gestation and declined from $23.5mm^2/cm^2$/10 days to $0.5mm^2/cm^2$/10 days.

The percentage of the term (140 days) placental growth (in terms of weight) that is achieved at 10 day intervals during pregnancy is shown in Figure 7-3.

Fig. 7-2: The total placental weight (a) and area (b) from triplet (o), twin (●) and singleton (□) pregnancies. A line has been drawn for the data from the twin pregnancies.

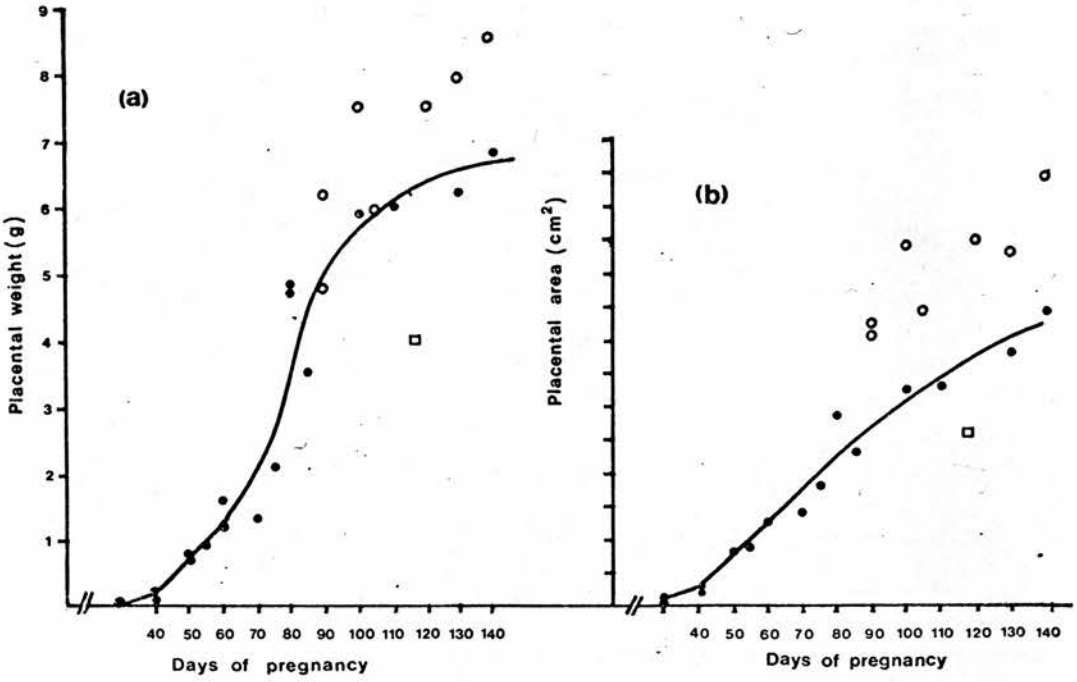
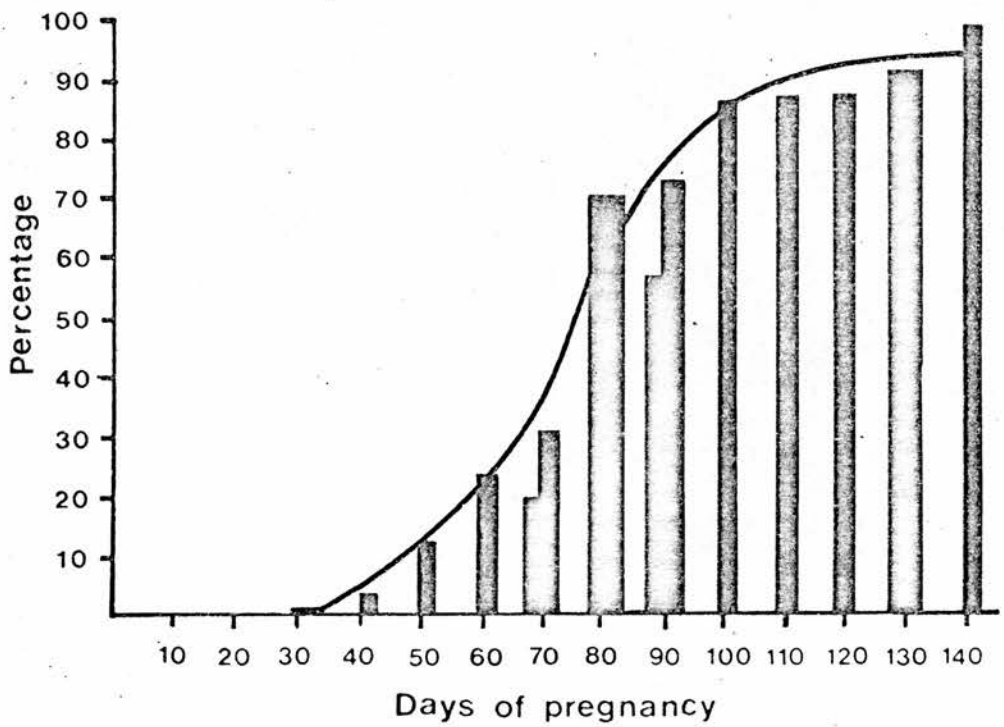


Fig. 7-3: The percentage of the 140 day placental weight that is achieved at 10 day intervals from 30 days of pregnancy. A triplet and twin pregnancy each were obtained at 140 days and the percentage for each triplet or twin pregnancy was calculated using the appropriate triplet or twin 140 day placental weight.



50% of its final growth was achieved between 70 and 80 days of pregnancy and nearly 90% by 100 days.

(c) Fetal weight

Fetal weight is shown in Figure 7-4. Twins were heavier than triplets after 130 days of gestation. A weight difference between triplets, twins and singletons was also found at birth. The body weight increased throughout pregnancy with a relatively steeper incline after day 100. The fetal growth was greatest in the last 10 days of pregnancy, increasing from 0.65g /10 days between day 70 and 80 to 6.64g /10 days between day 130 and 140. The rate of weight gain declined approximately 15 fold with advancing pregnancy, from 4850mgm/gm/10 days to 320mgm/gm/10 days.

The percentage of the term (newborn) fetal weight that is achieved at 10 day intervals during pregnancy is shown in Figure 7-5. Nearly two thirds of pregnancy is occupied in achieving the first 10% of the ultimate weight. The achievement of each succeeding decile in growth takes 10 days or less and 50% of the ultimate weight is achieved between 120 and 130 days.

The specific growth velocity (α) of the fetal marmoset was calculated using the generalised mathematical formula $W^{1/3} = \alpha(t-t_o)$. (Huggett and Widdas, 1951) The cube root of the fetal weight ($W^{1/3}$) is plotted against the fetal age (t) to give a regression line with the slope (α) and intercept (t_o) on the time axis. The data are plotted in Figure 7-6 and the formula obtained was $W^{1/3} = 0.039(t-56)$.

Fig. 7-4: The mean body weight (grams) of fetuses and newborns from triplet (o), twin (●) and singleton (□) pregnancies. B = birth.

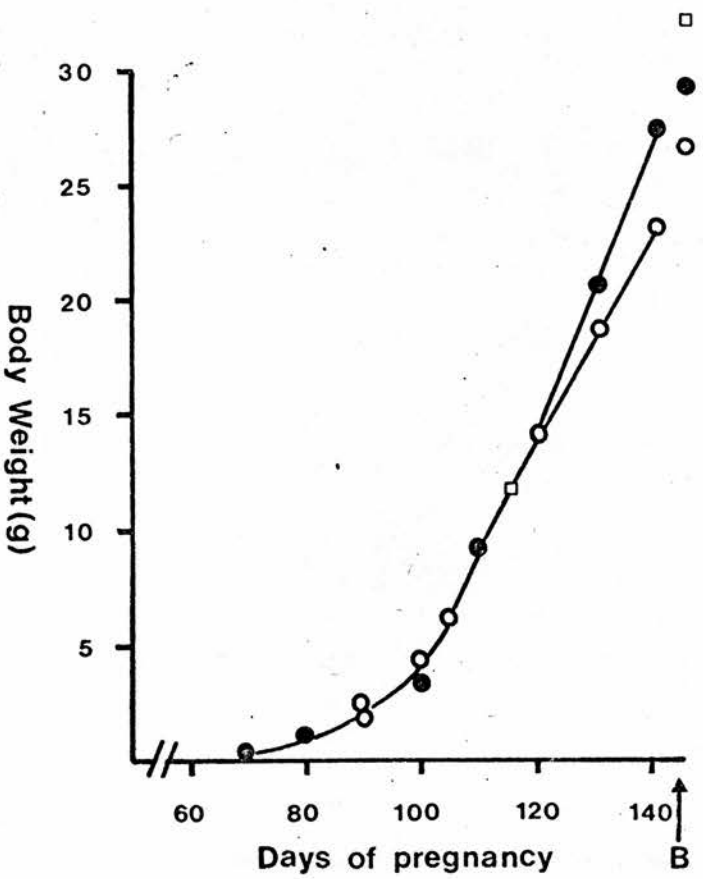


Fig. 7-5: The percentage of the newborn body weight that is achieved at 10 day intervals during pregnancy. The percentages for triplets and twins were calculated using the appropriate triplet or twin birth body weight.

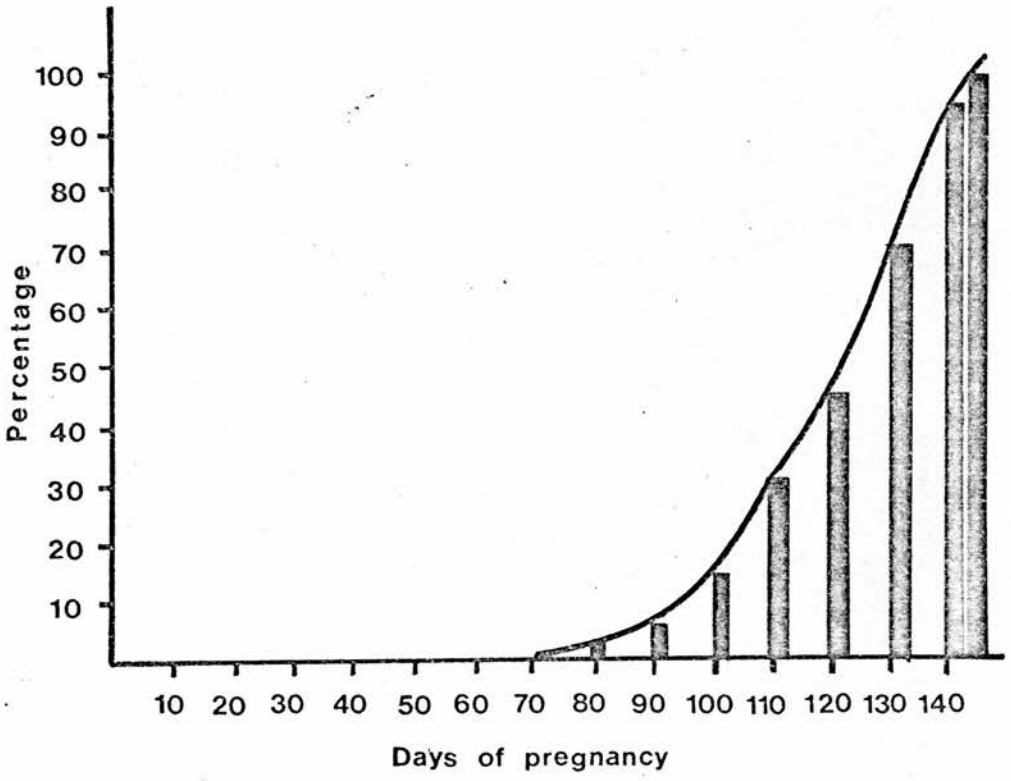
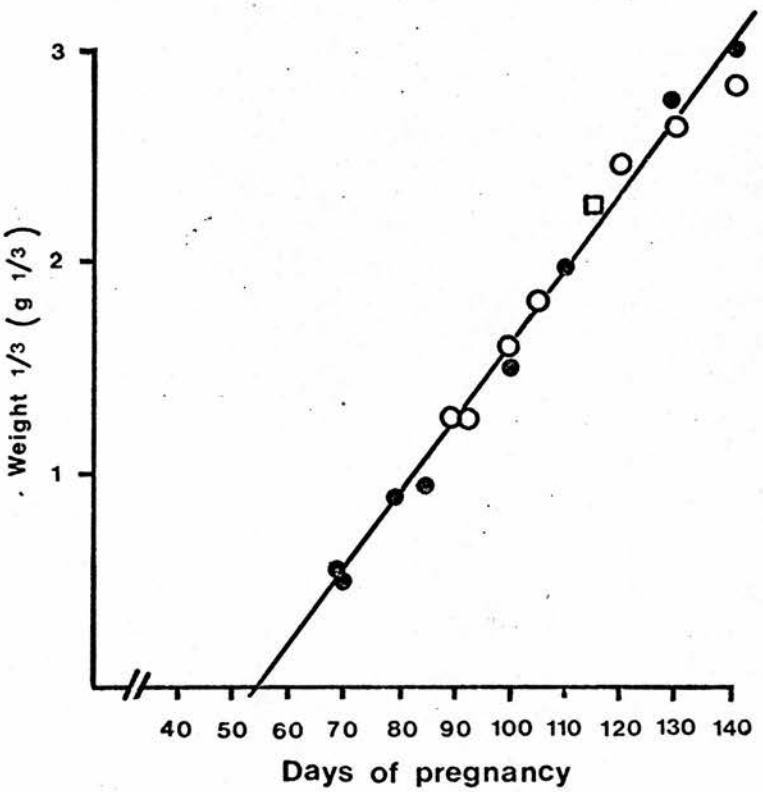


Fig. 7-6: The relationship between the cube root of the fetal weight (grams) and the stage of pregnancy. Triplet (○), Twin (●) and Singleton (□).



The correlation coefficient was 0.99. (between $W^{1/3}$ and t)

(d) Relationship between fetal and placental growth

The placental to fetal weight ratio is shown in Figure 7-7a. The ratio was highest (5:1) at day 70, fell precipitously to 1:1 at day 90 and to less than 0.5:1 after day 100. The ratio declined more slowly during the latter part of gestation and the placenta was about one tenth of the total fetal weight at day 140.

The weight of the placenta per fetus (Figure 7-7b) was greater for singleton pregnancies than twin and greater for twin pregnancies than triplet pregnancies.

The more rapid initial growth of the placenta than the fetus was also shown by the fact that the placenta reached 50% of its final weight at the same time (about day 80) as the fetus reached only 3% of its final weight. (Compare Figure 7-3 and 7-5). Likewise, the slowing of the placental growth at the end of pregnancy was more marked because, while the fetus achieved 90% of its final weight about 1 week before term (day 130), the placenta achieved nearly 90% of its final weight about 30 to 40 days before term.

(e) Organ weights

Table 7-3 gives the mean organ weight from each timed pregnancy from 80 to 140 days of gestation. The mean birth and adult organ weights are given in Table 7-4. There was an increase in organ weight with increasing fetal age and weight. With the exception of the brain and lungs,

Fig. 7-7: (a) The ratio between the total placental weight and the total fetal weight in triplet (o), twin (●) and singleton (□) pregnancies.
(b) The weight (grams) of placenta per fetus for triplet (o), twin (●) and singleton (□) pregnancies.

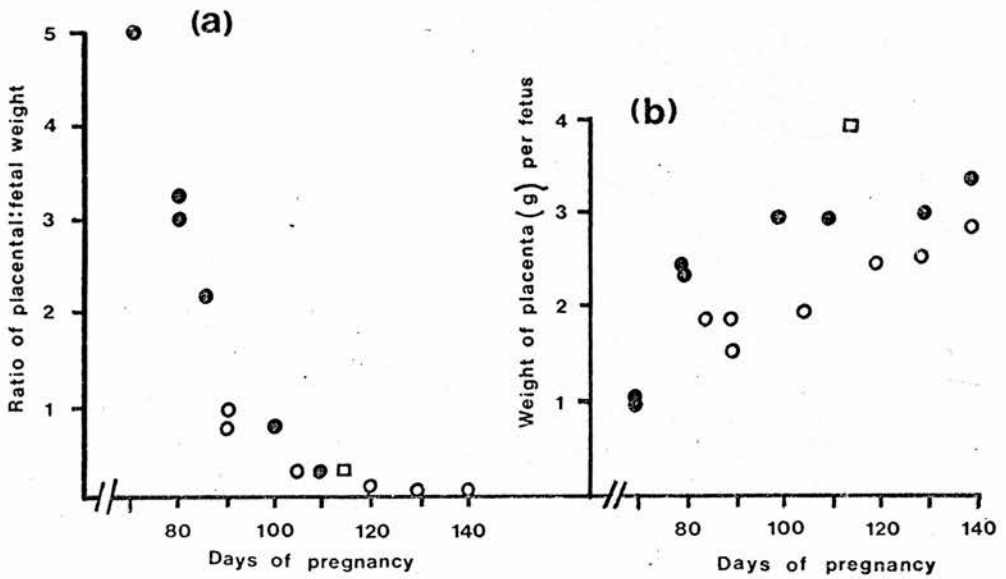


Table 7-3. The mean fetal organ and body weights (grams) from timed twin (TW) and triplet (TR) pregnancies from 80 to 140 days of gestation

Organ	Day of Gestation							
	80	90		100	110	120	130	140
	TW	TR	TR	TR	TW	TR	TW	TW
Brain		0.267	0.277	0.747	1.508	2.042	2.772	3.165
Liver		0.078	0.073	0.218	0.354	0.617	1.015	1.340
Lungs	0.0043	0.040	0.036	0.113	0.261	0.304	0.338	0.461
Thymus		0.002	0.003	0.005	0.032	0.038	0.059	0.113
Heart		0.015	0.020	0.061	0.131	0.153	0.208	0.186
Spleen		0.001	0.001	0.001	0.003	0.009	0.014	0.022
Adrenals	0.003	0.020	0.016	0.032	0.051	0.068	0.047	0.074
Kidneys		0.004	0.004	0.014	0.051	0.068	0.137	0.150
Testes	0.001	0.007	0.0069	0.0079	-	0.0064	0.0136	0.0126
Ovaries	0.0006	0.001	0.0027	0.0029	0.0047	0.0022	0.0038	0.0083
Fetus	1.10	2.10	2.07	4.47	9.22	14.62	20.78	27.42

Table 7-4. The mean (± s.d) birth (N = 8) and adult (N = 14) organ and body weights (grams).

Organ	Birth	Adult
Brain	3.817 ± 0.240	7.12 ± 0.44
Liver	1.633 ± 0.489	15.2 ± 5.4
Lungs	0.557 ± 0.178	2.68 ± 0.59
Thymus	0.076 ± 0.0245	-
Heart	0.334 ± 0.085	1.84 ± 0.43
Spleen	0.027 ± 0.006	0.324 ± 0.157
Adrenals	0.091 ± 0.014	0.1816 ± 0.064
Kidneys	0.191 ± 0.063	1.682 ± 0.54
Testes	0.0147 ± 0.0033	0.660 ± 0.184
Ovaries	0.00687 ± 0.002	0.076 ± 0.022
Fetus	32.3 ± 4.2 (S) (N=20) 29.3 ± 3.0 (TW) (N=226) 26.8 ± 3.0 (TR) (N=122)	287 ± 46 (N=64)

the largest increase in fetal organ weight increments was between 120 and 140 days of gestation. The maximum weight increment in brain and lung growth was at 100 to 110 days. In contrast, the maximum growth rate was always during the earliest period measured, which was 80 to 100 days of gestation.

The percent of the total body weight accounted for by each organ (relative growth rate) is given in Table 7-5. The liver was about the same proportion of the total body weight throughout fetal life. The spleen and thymus showed an increase and the gonads and adrenals a decrease in their relative growth rate. The relative growth rate of the brain, lungs and heart decreased after 110 days of gestation. Comparison of the birth and adult percentage showed a marked decrease for the brain and adrenals, and an increase for the testes. The percentage generally decreased for the other organs.

Table 7-6 shows the percentage of each organ's birth weight that is achieved between 80 and 140 days of gestation. Generally, 50% of the birth weight was reached by 120 to 130 days of gestation, the same period at which 50% of the fetal body weight was reached. The adrenals and gonads were 50% of their birth weight relatively earlier in gestation, between 100 and 110 days of pregnancy. Most organs and the total body weight had accomplised 10-20% of the final adult weight by full-term gestation. The exceptions were the brain and adrenals, which were more than 50% of their full adult size, and the testes which were only 2%.

Table 7-5. The percent of the total body weight accounted for by each organ (relative growth rate) from 90 to 140 day of gestation and at birth and in adults.

	Day of Gestation							
Organ	90	100	110	120	130	140	Birth	Adult
Brain	13.34	16.72	16.34	13.97	13.34	11.54	13.02	2.48
Liver	3.52	4.87	3.84	4.22	4.88	4.89	5.57	5.30
Lung	1.71	2.53	2.83	2.08	1.63	1.68	1.90	0.93
Thymus	0.14	0.11	0.35	0.26	0.28	0.41	0.25	-
Heart	0.98	1.36	1.41	1.04	1.00	0.68	1.13	0.64
Spleen	0.01	0.02	<0.03	0.06	0.07	0.08	0.09	0.11
Adrenals	0.77	0.72	0.55	0.47	0.66	0.39	0.31	0.06
Kidneys	0.18	0.32	0.56	0.47	0.66	0.39	0.65	0.59
Testes	0.333	0.177	0.044	-	0.065	0.046	0.050	0.230
Ovaries	0.130	0.065	0.051	0.015	0.018	0.030	0.023	0.026

Table 7-6. The percentage of each organ's birth weight achieved at various stages of gestation and the percentage of the birth to adult (B/A) organ weight. The mean, (±s.d.), excluding the gonads, is given.

	Days of Gestation							
Organ	80	90	100	110	120	130	140	B/A
Brain		7.2	19.6	39.5	53.4	72.6	82.9	53.6
Liver		4.5	13.3	21.6	37.8	62.1	82.0	10.7
Lungs	0.7	6.5	19.8	45.7	53.3	59.2	81.0	20.7
Thymus		4.0	6.6	42.1	50.0	77.6	148	-
Heart		6.0	18.3	34.2	54.8	62.2	55.6	18.1
Spleen	6.3	3.7	3.7	11.1	33.3	51.8	81.5	8.3
Adrenals	3.8	17.5	35.6	56.0	74.7	51.6	81.3	50.1
Kidneys	2.1	7.3	26.7	35.6	35.6	71.7	78.5	11.4
Testes	6.8	46.9	53.7	-	43.5	92.5	85.7	2.2
Ovaries	9.5	39.3	42.2	68.4	32.0	55.3	120.8	9.0
Mean	3.2	7.1	18.0	36.4	48.0	63.6	86.4	
±s.d.	2.4	4.4	10.3	14.0	13.3	9.6	26.5	
n	4	8	8	8	8	8	8	

(f) Body dimensions

(i) Crown-rump length

The crown-rump length (CRL) from day 50 to 140 of pregnancy and at birth is given in Figure 7-8. There was very little difference (less than 3%) between the crown-rump length of fetuses from twin and triplet pregnancies. The difference was not significant at birth. The maximum growth increments were found between 70 and 110 days of gestation. The mean increase during this time period was 1.2 $\pm$ 0.15cm/10 days (s.d.). Between 50 and 70 days and between 110 and 140 days, the increment in growth was from 0.38 to 0.70cm/10 days. The rate of growth is greatest early in gestation. There was approximately a 25 fold decline in the rate from 14.7mm/cm/10 days at 50 to 60 days to 0.63mm/cm/10 days at 130 to 140 days.

There was a linear relationship between the CRL and the cube root of the fetal weight ($W^{1/3}$). The regression line was $y = 0.38x + 0.09$ ($y = W^{1/3}$, x = CRL) and the correlation coefficient was 0.95 (n=15).

(ii) Trunk, limb and head measurements

The trunk, limb and head measurements from 90 to 140 days of pregnancy are given in Table 7-7. The birth and adult measurements are given in Table 7-8. All fetal dimensions increased with advancing gestation. The maximum increment in growth per 10 day interval was found between 90 and 120 days of gestation in all 20 actual measurements and in the 3 calculated measurements. In 10 of

Fig. 7-8: The mean crown-rump length (CRL) of fetuses and newborns from triplet (o), twin (●) and singleton (□) pregnancies. B = birth.

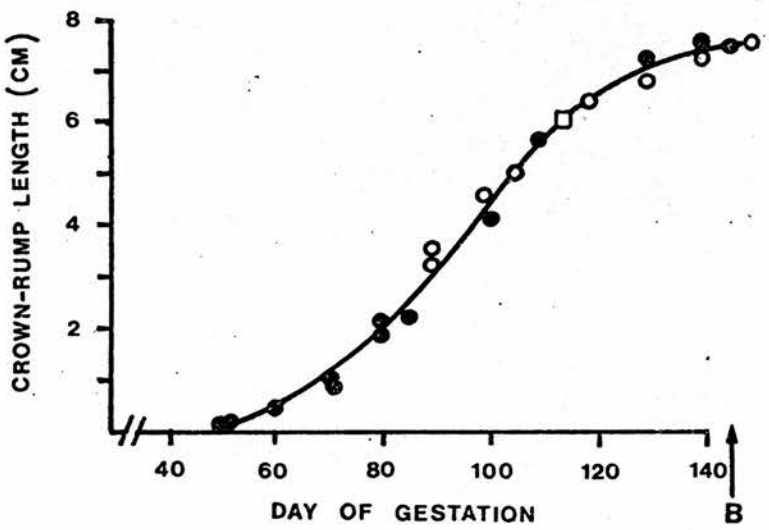


Table 7-7. The mean trunk, limb, head and tail measurements (cm) in fetal marmosets from 90 to 140 days of gestation from timed twin (TW) and triplet (TR) pregnancies. * gives a composite measurement calculated from individual measurements (see Table 7-1 for abbreviations). The pregnancy at 100 days consisted of 2 apparently normal and 1 abnormal fetus. The mean of the 2 normal fetuses is given on the left and of the abnormal fetus, on the right.

	Day of Gestation						
Measurement	90 TR	TR	100 TR	110 TW	120 TR	130 TW	140 TW
TRUNK							
C.R.L.	3.50	3.35	4.57; 2.70	5.85	6.42	7.25	7.70
T.H.	1.85	1.90	2.70; 1.62	3.42	3.77	4.27	4.70
S.B.	0.83	0.84	1.17	1.50	1.83	1.90	2.05
H.B.	0.48	0.50	0.75	0.90	1.06	1.25	1.45
C.B.	-	0.62	0.75	1.01	1.15	1.30	1.45
C.D.	-	0.63	0.80	1.08	1.29	1.49	1.58
C.C.	-	2.10	3.20	4.60	4.60	4.95	5.10
LIMB							
T.L.	0.68	0.67	1.07; 0.70	1.40	1.67	1.95	2.15
K.H.	0.62	0.61	1.07; 0.65	1.31	1.67	1.95	2.27
L.L.	-	0.54	0.99	1.10	1.53	1.75	2.10
F.L.	0.61	0.60	1.05	1.50	1.97	2.29	2.60
U.A.L.	0.73	0.73	1.12	1.45	1.65	1.98	2.50
F.A.L.	0.65	0.66	1.00	1.32	1.55	1.80	2.05
H.L.	0.43	0.46	0.84	1.20	1.60	1.64	1.67
*U.L.L.	1.81	1.85	2.36	3.97	4.80	5.42	5.79
*L.L.L.	1.30	1.28	2.14; 1.35	2.71	3.34	3.90	4.42
HEAD							
H.W.	0.90	0.93	1.23; 0.68	1.51	1.63	1.80	2.02
H.L.	0.19	1.18	1.55; 0.88	1.90	2.30	2.67	2.77
H.H.	0.90	0.90	1.13	1.15	1.45	1.66	1.65
T.F.H.	-	0.41	0.50	0.75	0.83	0.90	1.00
U.F.H.	-	0.30	0.37	0.45	0.53	0.58	0.60
*A.H.D.	1.00	1.00	1.30	1.52	1.79	2.04	2.14
Tail length		1.91	3.70	5.75	7.23	8.70	9.70

Table 7-8. The (±s.d.) trunk, limb, head and tail measurements (cm) in newborn marmosets (N = 8; Day 1) and adults (N = 12). * gives a composite measurement calculated from individual measurements (see Table 7-1).

Measurement	Birth	Adults
TRUNK		
Crown-rump	7.70 ± 0.40	20.36 ± 0.60
Trunk height	4.81 ± 0.41	12.54 ± 0.91
Shoulder breadth	2.40 ± 0.10	4.66 ± 0.23
Hip breadth	1.52 ± 0.07	3.73 ± 0.16
Chest breadth	1.65 ± 0.07	3.95 ± 0.29
Chest depth	1.61 ± 0.08	4.32 ± 0.35
Chest circumference	5.50 ± 0.80	12.62 ± 1.31
LIMB		
Thigh length	2.18 ± 0.10	6.45 ± 0.32
Knee heel	2.36 ± 0.11	6.60 ± 0.21
Leg length	2.17 ± 0.08	6.18 ± 0.27
Foot length	2.83 ± 0.12	5.56 ± 0.27
Upper arm length	2.15 ± 0.09	5.31 ± 0.21
Forearm length	2.00 ± 0.08	4.89 ± 0.26
Hand length	1.80 ± 0.12	3.76 ± 0.18
* Upper limb length	5.95	13.96
* Lower limb length	4.54	13.05
HEAD		
Head width	2.00 ± 0.08	3.15 ± 0.82
Head length	2.93 ± 0.06	4.29 ± 0.10
Head height	1.85 ± 0.10	2.00 ± 0.15
Total face height	1.01 ± 0.20	1.68 ± 0.53
Upper face height	0.61 ± 0.10	1.08 ± 0.12
* Average head diameter	2.26 ± 0.08	3.15 ± 0.07
Tail length	9.9 ± 1.01	25.3 ± 1.9

of the 20 actual measurements and in all 3 of the calculated measurements, the maximum increment was found between day 90 and 100. The growth increment from 130 to 140 days of gestation was a mean of 48.1 ± 6% (s.e.m. n=20) of each measurement's own maximum growth increment.

A combination of smaller growth increments and increasing body size with advancing gestation resulted in a marked decline in the rate of growth between 90 and 140 days of gestation. The mean decline in the rate was 6.1 ± 2 (s.d.) fold between 100 and 140 days.

Table 7-9 shows the mean percentage of the trunk, limb and head birth body dimensions that was achieved at 10 day intervals from day 90 to 140. By 100 days of gestation, the fetal body dimensions achieved about one half of their birth size. The head measurements reached a larger percentage of their birth size earlier in gestation and at birth was substantially larger compared to its ultimate adult size than the trunk or limb measurement.

7.4 Discussion

(a) Standards of growth

This chapter gives the first report on normal prenatal growth and development in the marmoset which utilizes material from pregnancies in which the stage of gestation was accurately determined by the post-ovulatory rise in progesterone.

Table 7-9. The mean (±s.d.) percentage of the ultimate birth size achieved at 10 day intervals during fetal life and the percentage of the birth to adult growth. The trunk, limb and head measurements consist of the mean of 6, 6, and 5 individual measurements respectively, which are listed in Table 7-1. The overall mean percentage for each 10 day interval and at birth is given. The tail is excluded since it is the only non-composite figure.

Measurement	90	100	110	120	130	140	Birth/Adult
Trunk	36 ± 2	51 ± 5	69 ± 8	76 ± 5	82 ± 8	95 ± 6	41 ± 5
Limb	28 ± 5	47 ± 5	62 ± 6	75 ± 6	86 ± 4	97 ± 5	41 ± 7
Head	45 ± 4	57 ± 5	68 ± 7	79 ± 2	90 ± 2	95 ± 5	68 ± 11
Tail	19	37	58	73	88	98	39
Mean (excl tail)	37 ± 8	52 ± 5	67 ± 4	77 ± 2	86 ± 4	96 ± 1	50 ± 15

Probably the most commonly used and easily measured parameters of fetal growth are the fetal weight and crown-rump length (Watts, 1977). The present study indicated a clearcut demarcation in these measurements for 10 day intervals of pregnancy, a linear relationship between the 2 measurements, and a linear relationship between the weight (to the $\frac{1}{3}$ power) and gestational age. Consideration should be given to the number of fetuses present for later pregnancy for the weight measurements. Although crown-rump measurements showed considerable variability with the developmental horizon during early pregnancy in the human (Streeter, 1951; Nishimura, Takano, Tanimura and Yasuda 1968), crown-rump measurements in the marmoset were similar in timed pregnancies from the same stage of gestation. Combined with a study of the external characteristics, these parameters should prove to be a fairly accurate guide to the stage of gestation.

The value of having a standard of prenatal growth that can be easily utilised by other investigators has already been evident in this thesis. There is considerable literature on various aspects of marmoset development but other than stating early, mid and late pregnancy, it was not possible to interrelate the various studies or to relate them to the new work being done on the marmoset. Application of these standards allowed data on adrenal and gonadal development, embryonic and placental development, to be correlated to a gestational age and related to the hormonal changes occurring during pregnancy as outlined

in this thesis. Furthermore, gonadal, adrenal and placental tissue was preserved from the timed pregnancies taken in this study for future histological examination to supplement the existing observations.

(b) Placental and fetal development

In all cases in the present study, 2 placental discs of similar size and weight were found whether there were 1, 2 or 3 fetuses. The presence of 2 placental discs nearly equal in size, even in the case of a singleton, was suggested by Hampton (1975) to be the result of the large blastocyst enlarging and completely filling the uterine lumen before invasion of the uterine tissue begins. Fusion of the placental discs must occur during the initial stages of development as there were only 2 discs even in the case of triplets. The present study also confirmed previous reports of the embryos being situated in a common exo-coelom enclosed by a common chorion. Chorionic fusion of the twin placenta was observed in at least 3 species of the Callitrichidae - The Golden Lion marmoset (Leontocebus rosalia) (Benirschke and Layton, 1969), the Geoffroy 's tamarin (Oedipomidas geoffroyi) (Wislocki, 1932; 1938) as well as the common marmoset (Callithrix jacchus) (Hill 1926, 1932; Hill and Hill 1927). Chorionic fusion occured very early in gestation, prior to the bilaminar disc stage (Hill, 1932), which is earlier in pregnancy than any of the stages examined in this study ($<$30 days of pregnancy).

Fetal attachments are generally reported to be either with both fetuses attached to the same placental disc, which presumably was the result of both blastocysts implanting side by side, or with fetuses attached to different placental discs, which resulted from each blastocyst implanting on a different uterine surface (Wislocki, 1929; Hampton, 1975; Phillips, 1976). Similar findings were made in the present study but in addition, some fetuses also appeared to be attached to both placental discs. Interplacental and even interfetal attachments of the umbilical vein after an initial surface connection on the fetal surface of the placenta were previously reported by Hampton (1975). The data in the present study encompassed more pregnancies from later stages which may account for the present observation.

The marked abnormal development of one of the three fetuses at 100 days of gestation is not an unusual finding. Similar observations were reported by other authors and in our own colony of animals (Wislocki, 1939; Hampton and Hampton, 1965) (S. F. Lunn, per comm). Fetal death and resorption of the fetus was also indicated by the finding of blood chimerism in isosexual twins and in single born animals. Presumably a second or third embryo, opposite in sex to the other 2, died and was resorbed after contributing hemopoietic stem cells to its littermates (Gengozian, Batson, Greene and Gosslee, 1969; Gengozian, 1971; Gengozian and Patton, 1972).

(i) Fetal and placental weight

This study showed that unlike the human, there was no slowing down of growth in the last weeks of gestation, which resulted in a sigmoid growth curve for the human fetus. (Hendricks, 1964; Gruenwald, 1966; Brenner, Edelman and Hendricks, 1976). In fact, for the marmoset, the weight increments were greatest during the last 10 day interval sampled during pregnancy (day 130 to 140). Abbott (1979) has further shown that for the marmoset, the weight increments continue to increase for the first 100 days following birth.

In the human, a comparison of the pre- and post- natal growth curves showed a post-natal resumption of the linear period of growth that was seen from 30 to 36 weeks of fetal life (McKeown and Record, 1953; Tanner, 1978). Therefore, a restriction to fetal growth was implied during the latter weeks of pregnancy. Several factors were suggested to influence fetal growth (reviews include: Dawes, 1976; McKeown, Marshall and Record, 1976); one factor being the placental size. There was a correlation between placental and fetal weight for the human (Dawes, 1968; Aherne, 1966) and experimental reduction of the placental mass in sheep caused a reduction in fetal weight. (Alexander, 1964.)

In the marmoset, there was not sufficient data to correlate placental and fetal weight but like the human, (McKeown and Record, 1952) there was less placenta available to a triplet fetus than a twin, and triplet

fetuses weighed less than twins. The relative placental-fetal growth pattern was also similar to the human and rhesus as the ratio declined with advancing gestation and a greater percentage of the placental birth growth was achieved earlier in gestation than the fetal growth. However, at birth, the placental-fetal ratio in the marmoset (1:10) was lower than in the human (1:5) (Hendricks, 1964; Aherne, 1966) and rhesus (1:3) (van Wagenen and Catchpole, 1965; Kerr, Kennan, Waisman and Allen, 1969; Kerr, Allen, Scheffler and Couture, 1974) and it is likely there are additional factors which may be a better index of placental function (McKeown et al, 1976). These other factors may well be of greater relevance but are more difficult to assess and for the present, the weight relationship remains the main means of interspecies comparison.

(ii) Fetal growth velocity

The majority of mathematical formula relating fetal weights to gestational age involve a linear relationship between time (t) and a cube or higher root (n) of the fetal weight (W). Growth in several species follows nearly a cube-root relationship. Formula of the nature $W^n = \alpha(t-t_o)$ or $W^{1/3} = \alpha(t-t_o)$ are used for interspecies (Huggett and Widdas, 1951; Payne and Wheeler, 1967) and intraspecies (Spencer, Coulombe and van Wagenen, 1966) comparisons of fetal growth. The slope of the linear relationship (α) was termed the specific growth velocity (Huggett and Widdas, 1951), and characteristic values of α

were shown for particular species.

Comparison of the specific growth velocity of different mammalian fetuses showed that there was a clear-cut grouping of the primate, sub-primate and Cetacean species and that the marmoset fitted in with the proposed primate grouping (see Figure 7-9). The continuous lines on this graph were suggested to represent the limits of natural variation for land mammals. The present data suggested that perhaps this limitation should be slightly extended as calculation of the marmoset's specific growth velocity (0.039), although placing it within the primate grouping, was the lowest value of α yet reported. It should be noted that several of the values in this figure were calculated from the weight at birth and estimation of to and some errors may be caused by this estimation.

(iii) Fetal, birth and adult weight relationships

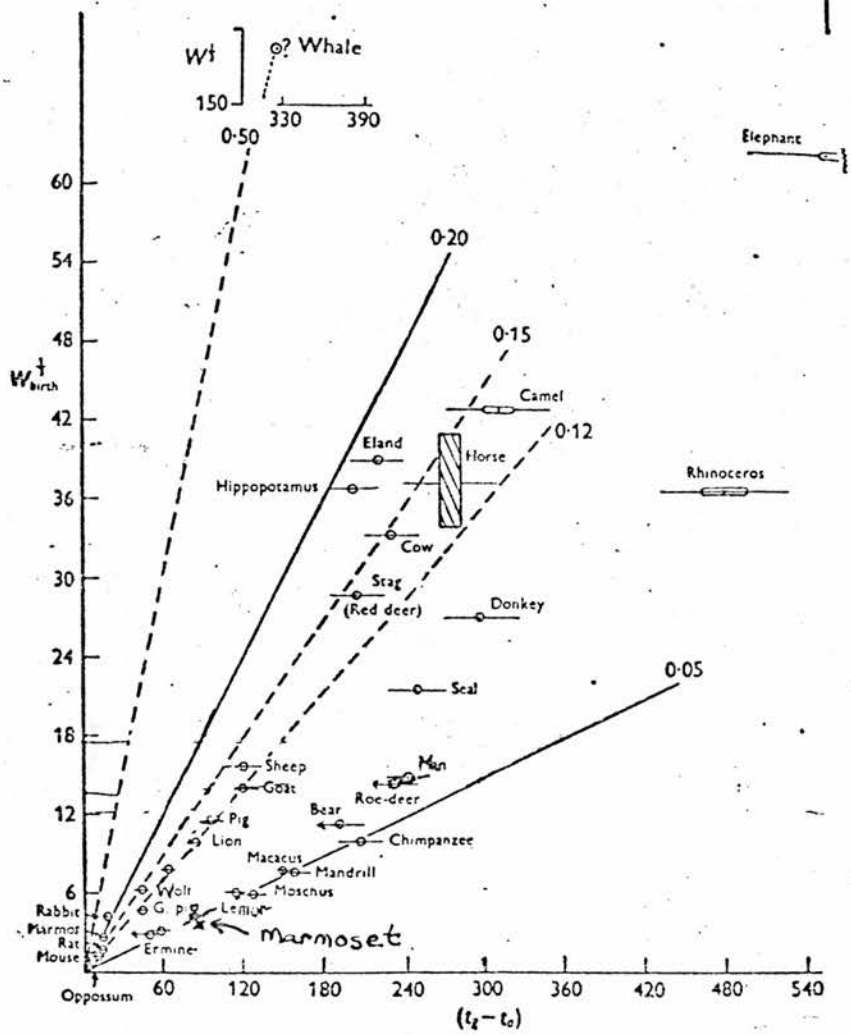
By birth, the marmoset was a percentage of its adult size which would be expected for a species of this size. The marmoset twin fetuses were a higher percentage (20%) of the maternal body weight compared to other primates (e.g. squirrel monkey: 16%, Goss, Popejoy, Fusiler and Smith, 1968; rhesus monkey: 8%; man: 6%; reviewed: Leitch, Hytten and Billewicz , 1959). This finding was to be expected as the logarithms of maternal and newborn weights have a straight line relation from bats to whales (Leitch et al, 1959) and even among a subgrouping of the anthropoid primates (Leutenegger, 1973). The marmoset was well within

Fig. 7-9: Plot of cube root of birth weight against gestation time less estimate of t_o. Continuous lines through the origin indicate the range of values of α (chart from Huggett and Widdas, 1951)

TABLE I. Formulae from published intrauterine weights and ages:

$Wt = 0{\cdot}102\ (t - 8)$	Mouse
$Wt = 0{\cdot}17\ (t - 11)$	Rat
$Wt = 0{\cdot}20\ (t - 12{\cdot}5)$	Rabbit
$Wt = 0{\cdot}09\ (t - 16)$	Guinea-pig
$Wt = 0{\cdot}147\ (t - 37)$	Sheep
$Wt = 0{\cdot}145\ (t - 50)$	Cow
$Wt = 0{\cdot}063\ (t - 33)$	Human

General formula $Wt = a(t - t_0)$



the normal range and as a general rule, the smaller sized species, have relatively larger newborns than larger sized species.

At birth, organs were a similar percentage of their adult weight in the marmoset as in the rhesus and human (rhesus: Kerr et al, 1969, 1974; Kerr and Waisman, 1970; human: Schulz, Griordamo and Schultz, 1962; Gruenwald and Minh, 1960 and others). In all these species the brain and adrenal were relatively large at birth. The relatively large size of the adrenal in these other species persisted only for a short time due to the involution of the 'fetal zone' following birth (Schulz et al, 1962). Benirschke and Richart's (1964) observations on the histology of the marmoset adrenal gland indicated a comparable explanation for the marmoset. Lanman (1957) confirmed the high adrenal-body weight ratio at birth in closely related tamarin species.

The actual percentage of the adult brain growth achieved by birth varied between species. The percentage for the marmoset (54%) was intermediate between the rhesus (68%) and the human (30%). How this overall interspecies comparison relates to the growth of the different parts of the brain, which have different functional significance, is only known from limited data on the human (Tanner, 1978).

Some reservations must be kept in mind regarding the organ weights. An organ may be difficult to separate from surrounding fat and connective tissue.

Fluid or blood may be sequestered in the lungs. In the present study, heart blood was removed for hormonal measurement. Small organ weights would be more susceptible to weighing errors. Whereas fetal organ weights were taken soon after hysterotomy, newborn and adult organ weights were generally taken an unknown time after death. Also, these animals generally died from unknown causes, and may not be entirely normal.

(iv) Trunk, limb and head measurements

Similar to the human (Tanner, 1978) and rhesus (van Wagenen and Catchpole, 1965), the peak in length gain preceeded the peak in weight gain in the marmoset. Also, by birth, the body dimensions were a larger percentage of their adult growth than the body and organ weights. However, like all other species, the maximum growth rate per unit of measurement was greatest at the earliest stages studied for both the body dimensions and body weights.

Possibly related to the relatively larger percentage of brain growth achieved by birth, was the relatively larger percentage of adult head size achieved by birth. This was a common finding among primates (Schultz, 1941, 1956). Head size may be an important factor in the successful delivery of the young. The relatively large cranial dimensions of the squirrel monkey (Leutenegger, 1970a,b) results in a difficult delivery (Bowden, Winter and Ploog, 1967; Hopf, 1967). Leutenegger (1973) suggested that the combination of the two factors of

a relatively large fetal to maternal size and relatively large head to body size in the marmoset resulted in newborn cranial dimensions that were quite large compared to the maternal pelvic inlet dimensions and constituted a strong reason for the production of multiple offspring in callitrichids. In other words, if singletons were regularly produced, the negative allometric relationship generally found for the above two factors would result in an oversized fetus, which would be difficult to deliver.

(c) Temporal relationships of prenatal development

It was previously suggested that the marmoset may have a lengthened embryonic period relative to other species (Phillips, 1976). Further analysis of the gonadal development data of Hampton and Taylor (1971) also indicated a relatively later onset of fetal sexual differentiation. (See Chapter 6). It was also suggested that there was not a sustained rise in progesterone and oestradiol in the peripheral plasma (Chapter 4) and that the placenta did not assume a major role in hormone production (Chapter 5) until later in gestation when compared to the human. The data in this chapter will be utilised to show that prenatal development is also slower during the first part of pregnancy compared to other primate species.

Comparison of the percentage of birth growth achieved at various stages of gestation showed that the marmoset did not achieve 50% of its birth size until relatively late in gestation (Figure 7-5 and Table 7-6,9). An interspecies comparison for the crown-rump length and

body weight is shown in Figure 7-10. Halfway through gestation, approximately day 70, the marmoset lags behind the growth achieved by these other species and it is not until more than three quarters of gestation is completed that the percentage becomes comparable. A preliminary observation on long bone growth in another New World primate, the squirrel monkey, suggested that this species may also achieve a lower percentage of its birth size compared to the rhesus monkey when 50% of gestation is completed (McKim, Hutchinson and Gavan, 1972).

Comparison among the primates in the initiation and duration of the embryonic period was presented by Hendrickx, Sawyer, Lasley and Barnes (1975). A summation of their data is given in Table 7-11. The embryonic period was considered to begin with the formation of the primitive streak and end with the initial ossification of the humerus. This data shows that although the marmoset has one of the shortest gestation lengths (only the galago was shorter) the embryonic period began and ended later in gestation, with respect both to the number of days of gestation and the percentage of gestation achieved. The embryonic period was also of substantially longer duration.

The lengthening of the embryonic period was probably a post-implantation phenomena as implantation was estimated to occur around day 10 in the marmoset (Hearn and Hodges per'l commun.), a similar time as in the human, rhesus and baboon. The primitive streak stage was approximately 15 to 20 days later in the marmoset (Phillips, 1976;

Fig. 7-10: The percentage of the birth crown-rump length (CRL) and the birth body weight (BWT) achieved 25, 50 and 75% through gestation. Man (●) (Streeter, 1920) rhesus (O) (van Wagenen and Catchpole, 1965), baboon (□) (Hendrickx, 1971) and marmoset (✱) (present study).

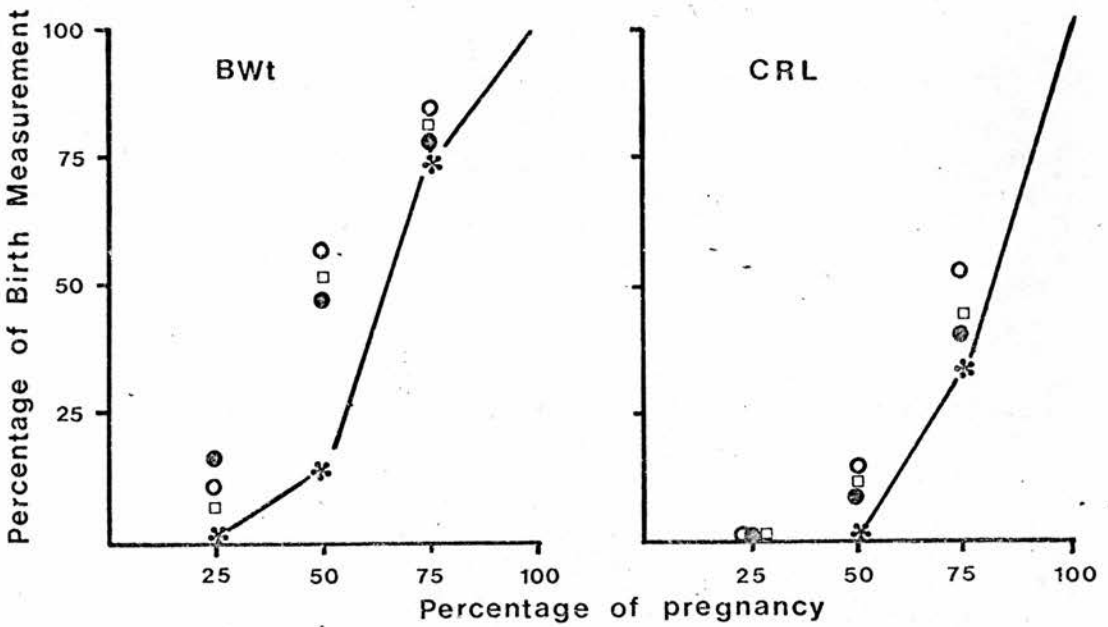


Table 7-11. The embryonic period (e.p.) in relation to the gestation length (G.L.) for several primate species. Date for all species except the marmoset was derived from Hendrickx et al, 1975; the marmoset data was obtained from Phillips (1976) and the present study.

Species	G.L.	beginning of e.p.		end of e.p.		Duration of e.p.
		day	% of gest. achieved	day	% of gest. achieved	
Galago	130	23	15	50	38	27
Green	155	21	13	48	31	27
Crab-eating	165	17	10	46	28	29
Bonnet	165	17	10	46	28	29
Rhesus	165	17	10	46	28	29
Baboon	180	17	9	46	16	29
Man	270	19	7	54	20	33
Marmoset	144	~30	~20	~80-85	~55	~50

present study) but less than 10 days later in these other species. Furthermore, comparison of the external characteristics of the marmoset embryo with the human, rhesus and baboon embryo indicated that the 60 day marmoset embryo resembled approximately the 29 to 31 day human embryo (Streeter, 1951) the 24 to 26 day rhesus embryo (Heuser and Streeter, 1941) and the 27 to 28 day baboon embryo (Hendrickx, 1971). This was approximately a 40 day interval from the primitive streak stage in the marmoset compared to about a 10 day interval in these other species. The 80-85 day marmoset embryo was similar to approximately the 50 day human, the 45 day rhesus and the 46 day baboon embryo and in this case, the interval between the latter 2 stages of embryonic development was approximately 20 days for all of the species.

Therefore the lengthened initiation and duration of the embryonic development period was probably primarily due to a lengthening of the earlier stages of development, which occurred between days 10 and 60 in the marmoset. A fairly similar conclusion was reached by Phillips (1976). Although he more accurately compared the stages of embryonic development by utilisation of the Streeter classification, he did not generally know the accurate gestation age associated with the developmental horizon.

The reason for the slower development of the marmoset embryo during the first part of gestation is unknown. It may be associated with the generally slower placental development which is found in New World monkeys compared to Old World monkeys (Hill, 1932; Beck, 1976;

Hampton, 1975; T. Baker, per'l commun.). A seasonal factor is probably irrelevant as young are produced throughout the year (Phillips, 1975).

A common finding is a delay in the prenatal development (e.g. delayed implantation) in species which ovulate shortly after birth and during lactation (reviewed Aitken, 1979). Although the marmoset is often lactating for the first 60-80 days of pregnancy (Lunn and McNeilly, 1981), there are significant differences between the marmoset and these species. Prenatal development in the marmoset is not halted, but merely slowed down and this occurs whether the mother is suckling young or not.

Phillips (1976) suggested that the lengthened embryonic period may be related to the unusual embryology of the marmoset. There may be delayed development during which time the twin chorionic cavities enlarge and fuse to become the common exo-coelom, which then undergoes a period of enlargement.

In summary, if comparable times in pregnancy are compared for prenatal development between species, the marmoset would lag behind other species for more than the first half of pregnancy. This factor should be remembered in interspecies comparisons of peripheral and fetal hormonal levels and of relative ovarian-placental function.

7.5 Chapter summary

1) Standards for prenatal growth were established for known gestational ages. These could be easily used by other investigators.

2) The marmoset apparently has a lengthened period of embryonic development compared to other primate species. The extension was due to prolonged development between 10 and 60 days of pregnancy, after which prenatal development proceeded at a similar rate to other species.

3) Overall, placental and fetal growth followed a similar pattern to other species: a) the growth rate was greatest at the earliest time period studied; b) maximum linear growth preceeded maximum weight growth; c) head and brain growth was accelerated compared to the rest of the organs and body; d) there was a linear relationship between the cube root of the weight and gestational age; e) the placental to fetal weight ratio declined with advancing gestation; f) Maximum placental growth preceeded maximum fetal growth.

4) Comparison of the birth size with the adult size showed that the marmoset had achieved a size at birth which would be expected of a primate species of its size.

Chapter 8

GENERAL DISCUSSION

The purpose of this study was to investigate the endocrinology of pregnancy in the marmoset monkey. The initial work provided essential detailed basic information on normal hormonal levels and interrelationships in the peripheral plasma during pregnancy and lactation (Chap 4). Radioimmunassays were used which had been adapted and fully validated for marmoset plasma (Chap 3). The later studies investigated ovarian and placental function (Chap 5), the fetal hormonal environment (Chap 6) and fetal development (Chap 7) at frequent intervals throughout gestation utilising samples from accurately dated pregnancies. The data in the various chapters was interrelated to provide an integrated picture during pregnancy.

This is the first such study for a New World primate species. The data obtained allowed comparison of this species with other Old World sub-human primate species and the human (See Chap 4-7) and provided a base for more applied studies in this species in the future. The marmoset is a suitable laboratory primate for studies in reproductive biology (Chap 1.5) and some of the parallels and divergencies between the marmoset and other species suggest it has considerable inherent scientific interest. The studies in this thesis were designed to investigate some of these aspects (See Chap

1.6). This chapter will further discuss the findings in this thesis in relation to these aspects, perhaps indicating some further areas of research required in this species.

Unlike other primate species so far studied, lactation does not interfere with the return to fertility in the marmoset. Why is there this difference between the marmoset and other primates? This study shows that, like other primate species, there is a clear association during lactation between high levels of prolactin and a maintained suckling stimulus. Ovulation and pregnancy occur soon after birth in both lactating and non-lactating animals and it appears therefore, that the marmoset is not susceptible to the inhibitory influences of suckling and/or prolactin on reproductive function Both of these are implicated as causal agents for inhibiting reproductive function in women, but which of these two components is the more important has yet to be resolved (McNeilly, 1979). Since the return to fertility is delayed in lactating women, the mechanisms controlling this inhibition of reproductive function have been of considerable interest for contraceptive research (Howie and McNeilly, 1979). The differences between the marmoset and women in their return to fertility following birth may limit the use of the marmoset in this field. However, further investigations on the role of prolactin and the factors controlling prolactin secretion, in the marmoset, may provide useful comparisons with the human; as this study suggested significant differences between the species in the role of prolactin in inhibiting reproductive function and in its relationship with oestradiol during pregnancy.

The marmoset perhaps has no need of a lactation-

induced delay in the return to fertility. In contrast to the human, chimpanzee and gorilla which have long lactation induced birth intervals (Short, 1980) marmoset gestation is relatively long for such a small mammal, the young are well developed at birth, weaned between 60-90 days, grow rapidly and are relatively independent of the mother by the time the next young are born, approximately 160 days later (Lunn & McNeilly, 1981). Data in the present study showed that a high percentage of marmosets are lactating during the first part of pregnancy and that marmoset gestation is relatively long because of the lengthening of the early stages of development. Although the causes of this lengthening do not seem to be due to lactation per se as it occurs in all pregnancies irrespective of the lactational status of the animals, the effect of this lengthening is that the heavy physical and nutritional demands of later pregnancy and the steep increase in progesterone and oestradiol values associated with increasing placental function are delayed until lactation is completed.

The lengthened period of early development is one of the striking differences between the marmoset and the human. The reasons for the delay in early pregnancy are not known (See Chap 7.4). However, differences in the timetable of prenatal development may not be an unusual finding and as more species are studied, an increasing range of interspecies differences may be found. From data already available in other mammalian species, it seems that there are 2 principal ways in which a delay in

prenatal development occurs; first, delayed implantation, which is a well-known phenomena depending on environmental or physiological conditions (reviewed: Aitken, 1979) and second, a generally slower rate of development, which was indicated for the galago (Hendrickx, et al, 1975) and possibly the squirrel monkey (Goss, et al, 1978; McKim et al, 1972). The present study confirmed, and in addition, defined the timing of the slow period of development in the marmoset. The marmoset is the first sub-human primate species in which the differences in the timetable or prenatal development were studied in conjunction with the hormonal events. These studies showed that the events which are contained within the first trimester of human pregnancy are spread out for more than one-half of pregnancy in the marmoset. This was seen in the hormonal profiles and ovarian-placental function as well as the prenatal development.

This study provided the first detailed information on the hormonal profiles of a wide range of hormones during pregnancy and showed that there were several similarities in the hormonal profiles and interrelationships to the human (See Chap 4.4). However, several salient differences were also indicated, the most notable of which include the very high oestradiol and oestrone levels, the reversal of the oestradiol to oestrone ratio during the first 2/3rds of pregnancy, and the significant decline in progesterone during the last weeks of pregnancy. This thesis related the hormonal profiles

to ovarian and placental function (See Chap 5 and below), but other factors relevant in mediating the peripheral plasma hormonal levels remain to be defined. These factors include the production and metabolic clearance rates, the amount of hormone conjugated, and the levels of steroid binding proteins. This latter factor will relate to how much of the measured hormone is actually found in the unbound form, which is considered to be the active form. Measurement of the unbound fraction of the steroid hormones in the marmoset would perhaps provide a better comparison with the human of the amounts of hormones actually available to the target tissues.

Ovarian and placental function were studied in timed pregnancies at frequent intervals throughout gestation, These studies establish the timing of the periods of ovarian and placental dominance and the timing of the transitional period from ovarian to placental dominance. The exact timing of the dispensability of the corpus luteum still needs to be determined for the marmoset by ovariectomies on timed pregnancies. The transitional period may be of interest for establishing whether there is a relationship between ovarian and placental secretion, and whether placental function is the same in normal pregnancies and in those in which there is an earlier than normal decline in ovarian function. In the marmoset, ovarian - placental function may be easily compared between normal animals and those which had been ovariectomised during the transitional period, using the <u>in vivo</u>

and in vitro techniques used in the present study. These techniques may also be used to assess ovarian-placental function in marmosets which become pregnant following immunisation against hormones (e.g. hCG and LHRH) (Hearn et al, 1975; Hodges and Hearn, 1978) which may affect either ovarian and/or placental function (See Chap 1.4). It may be of particular interest to assess ovarian - placental function in these immunised animals because when these animals returned to breeding, they experienced a series of recurrent abortion during the transitional period (Hearn, 1976).

The studies in this thesis on placental function also showed that the steep increase in peripheral plasma progesterone and oestradiol levels, initiated after day 90 of gestation, was related to the increasing placental function at this time. In this respect, the marmoset resembles the human more closely than does the macaque. The factors involved in regulating the increase in placental function, which occur in both the human and marmoset are not yet known. Some factors (See also Chap 1.4) that have been investigated include the role of the fetus, in particular for providing androgen percursors for placental aromatisation to oestrogen (Beling, 1977; Levitz and Young, 1977). In the marmoset, Ryan et al (1961) also showed the necessity for androgen precursors for placental oestrogen production but to what extent the fetus supplies the necessary androgen precursors is not known. There have also been some investigations on a fetal role

for placental progesterone production in the rhesus (Hagemenas and Kittinger, 1973, 1975; Lanman et al, 1975) but these studies have not generally been followed up. A role for oestrogen in maintaining placental progesterone secretion was suggested by Albrecht (1980), based on his studies in the baboon, and an interrelationship between placental CG, progesterone and LHRH was suggested by Wilson and co-workers (1980a,b), based on in vitro studies using human placental tissue.

This study showed that the marmoset has advantages for investigating some aspects of the control of placental steroidogensis. First, the increase in placental function is dramatic and is consistently associated with a precise stage of gestation and therefore only a short period of pregnancy need be studied. Second, the gestational changes in placental function were reflected in the in vitro secretion of progesterone and LH/CG in cultures of placental tissue. Placental and fetal tissues of a reasonable size for in vitro studies can be easily obtained during this period of gestation from timed pregnancies without interfering with an immediate return to breeding in the animal or without precluding its use for future hysterectomy procedures (Phillips, 1976; Hearn and Chambers, unpublished observations). The main disadvantage to the marmoset is that due to its small size, studies involving manipulative fetal surgery may be difficult.

This study found that the increasing placental

secretion of hormones which occurs after 90 days of pregnancy is also relected in the fetal compartment, and there is a substantial elevation in fetal hormonal levels. However, the changes in the fetal compartment are not necessarily parallel to those in the maternal compartment, the most obvious examples being the reversal of the oestrone to oestradiol ratio in the umbilical vein and fetal sera compared to the maternal plasma, and the increases in androgen levels which occur in the fetal compartment but not the maternal. The physiological significance of a reversal in the oestrogen ratio is not known although it is also found in the human and rhesus (See Chap 6.4).

The elevation in fetal testosterone levels occur both in female and male fetuses as a result of the increased placental secretion, and unlike the rhesus and human (reviews include: Reyes et al, 1976; Winter et al, 1977; Resko, 1977), there was not a distinct period of elevated testosterone levels due to a significant gonadal contribution in the male fetus compared to the female. Unless a short period of elevated testosterone levels, such as seen in the rat (Weisz and Ward, 1980), was missed, female fetuses apparently experience a similar androgen environment as males. The factors involved in sexual differention in the marmoset are as yet not completely understood (See also Chap 6.4.d). Perhaps a special arrangement exist in the marmoset which allows normal and independent development of male and female

fetuses despite their intimate relationship afforded by a common blood pool. Marmosets are unique among primates because of their high incidence of twinning and the presence of vascular anastomoses that occur between twin fetuses (Wislocki, 1939; Gengozian, 1971). Although this situation invariably results in haemopoeietic chimerism there is no evidence of freemartinism (Benirschke and Brownhill, 1962) or any masculinisation of the female co-twin. The freemartin question is beyond the scope of this discussion. Partial masculinisation of the female marmoset could be achieved during the early post-natal period by testosterone administration (Abbott, 1979), but whether females can also be masculinised in utero by testosterone administration is not known. The studies in this thesis showed that a critical period one might consider for androgen administration would be between 75 and 100 days of pregnancy. This is approximately from the time of the earliest sexual differentiation (Hampton and Taylor, 1971; see also Chap 6.4) to the time prior to the increase in circulating hormonal levels in the fetus. It may be important that the female fetus is not exposed to high androgen levels prior to this time. After 100 days, it may not matter that the female experiences high androgen levels but consideration may then be given to the effect of exogenous androgen on the progesterone to testosterone ratio, as there was a significant sex difference between male and female co-twins. Resko

(1977) suggested, based on studies in the rhesus, that this hormonal ratio may be important.

The studies on embryonic and fetal development in this thesis were the first in this species to be related to accurately dated pregnancies. The timing of the prenatal development patterns was accurately assessed and was compared with other species (See Chap 7.4). This study showed that although the marmoset has a lengthened period of early development, the fetal growth patterns during the latter half of gestation correspond to those that might be expected from a primate species of comparable size. Furthermore, the data provide a set of accurately timed growth standards for the fetal marmoset which can be utilised by other investigators.

In summary, the studies in this thesis fully describe the hormonal profiles and interrelationships in the peripheral and fetal plasma, the ovarian and placental function and the fetal development throughout pregnancy for the marmoset monkey. This is the first such study in a New World primate species. The studies showed that there are several similarities and differences to the human. One difference is that high levels of prolactin associated with suckling do not inhibit the return to fertility, whereas an important similarity is that the sustained increase in progesterone and oestradiol is related to increasing placental function. In the latter respect,

the marmoset more resembles the human than does the much used rhesus monkey, and therefore, in this respect the marmoset is a more useful primate model for studies during pregnancy than the rhesus. The gestational changes in ovarian and placental function were defined, and serve as a base for future investigations on this aspect of pregnancy, and some possible lines of research are described in this chapter. Measurement of fetal hormone levels showed that, unlike the human and rhesus, there is no definitive sex difference in hormone levels, and that the placenta is primarily responsible for the elevation in fetal hormonal levels. Finally, this study showed that a major difference between the marmoset and human is that there is a lengthened period of embryonic development in the marmoset, and that a comparable lengthening is seen in all aspects of pregnancy.

REFERENCES

Abbott D.H. (1979) Sexual development of the common marmoset, Callithrix jacchus jacchus. Ph.D. thesis dissertation. Edinburgh University.

Abraham G.E., Tulchinsky D & Korenman S.G. (1970) Chromatographic purification of oestradiol-17β for use in radio-ligand assay. Biochem. Med. 3, 365-368.

Abramovich D.R. (1974) Human sexual differentiation - in utero influences. J. Obstet. Gynaecol. Br. Commonw. 81, 448-453.

Abramovich D.R., Baker T.G & Neal P. (1974) Effect of human chorionic gonadotrophin on testosterone secretion by the fetal human testis in organ culture. Endocrinol. 60, 179-185.

Adams E.C. & Hertig A.T. (1969) Studies on the human corpus luteum. 11. Observations on the ultrastructure of luteal cells during pregnancy. J. Cell. Biol. 41, 761-735

Adcock E.W., Teasdale F., August C.S., Cox S., Meschia C., Battaglia F.C. and Naughton M.A. (1973) Human chorionic gonadotrophin: its possible role in maternal lymphocyte suppression. Science 181, 845-847.

Aherne W. (1966) A weight relationship between the human fetus and placenta. Biol. Neonate 10, 113-118.

Ahluwalia B., Williams J. & Verma P. (1974) In vitro testosterone biosynthesis in the human fetal testis. II Stimulation by cyclic AMP and human chorionic gonadotrophin (hCG). Endocrinology 95, 179-185.

Ainsworth L., Daenen M. & Ryan K.J. (1969) Steroid hormone transformations by endocrine organs from pregnant mammals. VI Biosynthesis and metabolism of oestrogens and progesterone by primate placental preparations in vitro. Endocrinology 84, 1421-1429.

Ainsworth L. & Ryan K.J. (1969) Steroid hormone transformations by endocrine organs from pregnant mammals. V The biosynthesis and metabolism of progesterone and oestrogens by orangutan placental tissue in vitro. Steroids 14, 301-314.

Aitken R.J. (1979) The hormonal control of implantation, in Maternal Recognition of Pregnancy. Ciba Fdn. Series 64 (new series) pp. 53-83, Excerpta Medica, Amsterdam.

Albrecht E.D. (1980) A role for oestrogen in progesterone production during baboon pregnancy. Am. J. Obstet. Gynaecol. 136, 569-576.

Albrecht E.D., Haskins A.L. & Pepe G.J. (1980) The influence of fetectomy at midgestation upon the serum concentrations of progesterone, oestrone, and oestradiol in baboons. Endocrinology 107, 766-770.

Albrecht E.D. & Townsley J.D. (1976) Serum progesterone in the pregnant baboon (Papio papio). Biol. Reprod. 14, 610-612.

Alexander G. (1964) Studies on the placenta of the sheep (Ovis aries L.). Placental size. J. Reprod. Fertil. 7, 289-305.

Allen W.M. (1953) In: Discussion of Hant A.F., McConakey W.M., Pregnancy associated with diseases of the adrenal glands. Am. J. Obstet. Gynaecol. 66, 970

Allen W.R. (1975) Endocrine functions of the placenta, in Comparative Placentation pp. 214-267, ed. D.H. Steven, Academic Press, London.

Anderson D.C. (1974) Sex-hormone-binding globulin. Clin. Endocrinol (Oxf) 3, 69-96.

Atkinson L.E., Hotchkiss J., Fritz G.R., Surve A.H., Neill H.D. & Knobil E. (1975) Circulating levels of steroids and chorionic gonadotrophin during pregnancy in the rhesus monkey, with special attention to the rescue of the corpus luteum in early pregnancy. Biol. Reprod. 12, 335-345.

Altmann J., Altman S.A. & Hausfater G.(1978) Primate infants effects on mothers future reproduction. Science 201, 1028-1030.

Baird D.T., Baker T.G., McNatty K.P & Neal P. (1975) Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. J. Reprod Fertil. 45, 611-619.

Baird D.T., Burger P.E., Heavon-Jones G.D. & Scaramuzzi R.J. (1974) The site of secretion of androstenedione in non-pregnant women. J. Endocrinol. 63, 201-212.

Baird D.T. & Fraser I.S. (1974) Blood production and ovarian secretion rates of estradiol-17β and oestrone in women throughout the menstrual cycle. J. Clin. Endocrinol. Metab. 38, 1009-1017.

Baird D.T., Swanston I. & Scaramuzzi R.J. (1976) Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the estrous cycle. Endrocrinology 98, 1490-1496.

Baker T.G. & Neal P. (1969) The effects of x-irradiation on mammalian oocytes in organ culture. Biophysik 6, 39-45

Bammann B.L., Coulam C.B., Jiang N. (1980) Total and free testosterone during pregnancy. Am. J. Obstet. Gynecol. 137, 293-298.

Barberia M., Abu-Fadil S., Kletzky O.A., Nakamura R.M. & Mishell D.R., Jr. (1975) Serum prolactin patterns in early human gestation. Am. J. Obstet. Gynecol. 121, 1107-1110.

Batra S. (1979) The regulation of oestradiol and progesterone concentrations in the female reproductive tissues. Acta Obstet. Gynecol. Scand. (Suppl) 82, 1-31.

Batra S., Bengtsson L.P., Grundsell H. & Sjoberg N.O. (1976) Levels of free and protein-bound progesterone in plasma during late pregnancy. J. Clin. Endocrinol. Metab. 42, 1041-1047.

Batzer F.R. (1980) Hormonal evaluation of early pregnancy. Fertil. Steril. 34, 1-13

Beamer N., Hagemenas F. & Kittinger G.W. (1972) Protein binding of cortisol in the rhesus monkey (Macaca mulatta). Endocrinology 90, 325-327.

Beck F. (1976) Comparative placental morphology and function. Environ. Health Perspect. 18, 5-12.

Bedford C.A., Challis J.R.G., Harrison F.A. & Heap R.B. (1972) The role of oestrogens and progesterone in the onset of parturition in various species. J. Reprod. Fertil. (suppl) 16, 1-23.

Behrman R.E., Parer J.T. & de Lannoy C.W. (1967) Placental growth and the formation of amniotic fluid. Nature 214, 678-680.

Beling C.(1977) Estrogens in The Endocrinology of Pregnancy pp. 76-98, eds. F. Fuchs, F. and A. Klopper, Harper and Row, London.

Bell E.T. & Loraine J.A. (1971) Hormone assays and their clinical application. Livingston, Edinburgh.

Benirschke K., Anderson J.M. & Brownhill L.E. (1962) Marrow chimerism in marmosets. Science 138, 513-515.

Benirschke K. & Brownhill L.E. (1962) Further observations on marrow chimerism in marmosets. Cytogenetics 1, 245-257.

Benirschke K. & Brownhill L.E. (1963) Heterosexual cells in testes of chimeric marmoset monkeys. Cytogenetics 2, 331-341.

Benirschke K. & Layton W. (1969) An early twin blastocyst of the golden lion marmoset, Leontocebus rosalia L. Folia Primatol. 10, 131-138.

Benirschke K. & Richart R. (1964) Observations on the fetal adrenals of marmoset monkeys. Endocrinology 74, 382-387.

Bird C.E., Wiqvist N., Diczfalusy E. & Solomon S. (1966) Metabolism of progesterone by the perfused previable human fetus. J. Clin. Endocrinol. Metab. 26, 1144-1154.

Blaquier J., Forchielli E. & Dorfman R.I. (1967) In vitro metabolism of androgens in whole human blood. Acta Endocrinol. (Copenh) 55, 697-704.

Bolte E., Mancuso S., Dray F., Baulieu E.E. & Diczfalusy E. (1964a) Conversion of testosterone-17α ^{3}H into 17β-estradiol-17α ^{3}H by human placentas perfused in situ. Steroids 4, 613-628.

Bolte E., Mancuso S., Eriksson G., Wiqvist N. & Diczfalusy E. (1964b) Studies on the aromatisation of neutral steroids in pregnant women. I. Aromatisation of C_{19} steroids by placentas perfused in situ. Acta Endocrinol. (Kbh) 45 535-559.

Bolte E., Mancuso S., Eriksson G., Wiqvist N. & Diczfalusy E. (1964c) Studies on the aromatisation of neutral steroids in pregnant women. II. Aromatisation of dehydroepiandrosterone and of its sulphate administered simultaneously into a uterine artery. Acta Endocrinol. (Kbh) 45, 560-575.

Bolte E., Mancuso S., Eriksson G., Wiqvist N. & Diczfalusy E. (1964d) Studies on the aromatisation of neutral steroids in pregnant women. III. Overall aromatisation of dehydroepiandrosterone sulphate circulating in the foetal and maternal compartments. Acta Endocrinol. (Kbh) 45, 576-599.

Borland R., Lake Y. & Wilson D. (1975) Immunological privilege resulting from endocrine activity of the trophoblast in vivo. In, Immunology of the Trophoblast, pp. 157-170, eds. R.G. Edwards, C. Howe and M.H. Johnson. Cambridge University Press, Cambridge.

Boroditsky R.S., Reyes F.I., Winter J.S.D. & Faiman C. (1975) Serum human chorionic gonadotropin and progesterone patterns in the last trimester of pregnancy: Relationship

to fetal sex. Am. J. Obstet. Gynecol. 121, 238-241.

Bosu W.T.K., Johansson E.D.B. & Gemzell C. (1973a) Peripheral plasma levels of oestrogens, progesterone and 17α-hydroxyprogesterone during gestation in the rhesus monkey. Acta Endocrinol. (Copenh) 74, 348-360.

Bosu W.T.K., Johansson E.D.B & Gemzell C. (1973b) Patterns of circulating oestrone, oestradiol-17β and progesterone during pregnancy in the rhesus monkey. Acta Endocrinol. (Copenh) 74, 743-755.

Bosu W.T.K. & Johansson E.D.B. (1974a) Ovarian morphology and the plasma levels of estrogens and progesterone in rhesus monkey in early pregnancy. Fertil. Steril. 25, 443-452.

Bosu W.T.K., Johansson E.D.B. & Gemzell C. (1974b) Influence of oophorectomy, luteectomy, foetal death and dexamethasone on the peripheral plasma levels of oestrogens and progesterone in the pregnant Macaca mulatta. Acta Endocrinol. (Copenh) 75, 601-616.

Bowden D., Winter P. & Ploog D. (1967) Pregnancy and delivery behavior in the squirrel monkey (Saimiri sciureus) and other primates. Folia primatol. 5, 1-42.

Braunstein G., Raser J., Adler D., Danzer H. & Wade M. (1976) Serum human chorionic gonadotropin levels throughout normal pregnancy. Am. J. Obstet. Gynecol. 126, 678-681.

Brenner W.E., Edelman D.A. & Hendricks C.H. (1976) A standard of fetal growth for the U.S. of A. Am. J. Obstet. Gynecol. 126, 555-564.

Bridgwater D.D. (1972) ed. Saving the Lion Marmoset. Proc. of the Wild Animal Propagation Trust, Golden Lion Marmoset Conference. Wild Animal Propagation Trust, West Virginia.

Brown J.B., Evans J.H., Beischer N.A., Campbell D.G. & Fortune D.W. (1970) Hormone levels in threatened abortion. Br. J. Obstet Gynaecol. 77, 690-

Bulletin on Sephadex LH-20 (1970), by Pharmacia Fine Chemicals. Uppsala, Sweden.

Butler W.R., Krey L.C., Lu K.-H., Peckham W.D. & Knobil E. (1975) Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. IV. Prolactin secretion. Endocrinology 96, 1009-1105.

Caligaris L., Astrada J.J. & Taleisnik S. (1974) Oestrogen and progesterone influence on the release of PRL in ovariectomized rats. J. Endocrinol. 60, 205-210.

Carpenter C.R. (1934) A field study of the behaviour and social relations of howling monkeys. Comp. Psychol. Monogr. 10, 1-68.

Carr B.R., Mikhail G. & Flickinger G.L. (1971) Column chromatography of steroids on Sephadex LH-20. J. Clin. Endocrinol. Metab 33, 358-360.

Castellanos H. & McCombs H.L. (1968) The reproductive cycle of the New World monkey. Gynecologic problems in a breeding colony. Fertil. Steril. 19, 213-227.

Catt K.J., Dufau M.L. & Vaitukaitis J.L. (1975) Appearance of HCG in pregnancy plasma following the initiation of implanation of the blastocyst. J. Clin. Endocrinol. Metab. 40, 537-540.

Cedard L., Alsat, E., Urtasum M. & Varangot J. (1970) Studies on the mode of action of luteinizing hormone and chorionic gonadotropin on estrogenic biosynthesis and glycogenolysis by human placenta perfused in vitro. Steroids 8, 361-375.

Challis J.R.G. (1971) Sharp increase in free circulating oestrogens immediately before parturition in sheep. Nature 229, 208.

Challis J.R.G. (1980) Endocrinology of late pregnancy and parturition. Int. Rev. Physiol. 22, 277-324..

Challis J.R.G., Davies I., Benirschke K., Hendrickx A. & Ryan K. (1974) The effects of dexamethasone on plasma steroid levels and fetal adrenal histology in the pregnant rhesus monkey. Endocrinology 95, 1300-1305.

Challis J.R.G., Davies I.J., Benirschke K., Hendrickx A.G. & Ryan K.J. (1974b) The concentrations of progesterone, estrone, estradiol-17β in the peripheral plasma of the rhesus monkey during the final third of gestation and after the induction of abortion with PGF_2a. Endocrinology 95, 547-553.

Challis J.R.G., Davies I.J., Benirschke K., Hendrickx A.G. & Ryan K.J. (1975) The effects of dexamethasone on the peripheral plasma concentrations of androstenedione, testosterone and cortisol in the pregnant rhesus monkey. Endocrinology 96, 185-192.

Challis J.R.G., Hartley P., Johnson P. , Patrick J., Robinson S. & Thorburn G.D. (1977b) Steroids in the amniotic fluid of the rhesus monkey (Macaca mulatta). Endocrinology 73, 353-363.

Challis J.R.G., Heap R.B. & Illingworth D.V. (1971) Concentrations of oestrogen and progesterone in the plasma of non-pregnant, pregnant and lactating guinea-pigs. J. Endocrinol. 51, 1333-1339.

Challis J.R.G., Kendall J.Z., Robinson J.S. & Thorburn G.D. (1977) The regulation of corticosteroids during late pregnancy and their role in parturition. Biol. Reprod. 16, 57-69.

Challis J.R.G., Robinson J.S. & Thorburn G.D. (1977b) Fetal and maternal endocrine changes during pregnancy and parturition in the rhesus monkey. in The Fetus & Birth pp 259-287, Ciba Fdn Symp 47; Excerp Med, Elsevier, N Holland

Chambers P.C. & Hearn J.P. (1979) Peripheral plasma levels of progesterone, oestradiol-17β, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey, Callithrix jacchus. J. Reprod. Fertil. 56, 23-32.

Chandrashekar V., Wolf R.C., Dierschke D.J., Sholl S.A., Bridson W.E. & Clark J.R. (1980) Corpus luteum function in the pregnant pigtailed monkey (Macaca nemestrina) (Abstract) Prog. End. Soc. 62nd. Meet. 1980.

Clarke I.J., Scaramuzzi R.J. & Short R.V. (1976) Effects of testosterone implants in pregnant ewes on their female offspring. J. Embryol. Exp. Morphol. 36, 87-99.

Clements J.A., Reyes F.I., Winter J.S.D. & Faiman C. (1976) Studies on human sexual development: III. Fetal pituitary, serum and amnictic fluid concentrations of LH,CG and FSH. J. Clin. Endocrinol. Metab. 42, 9-19.

Coe C.L. & Rosenblum L.A. (1978) Annual reproductive strategy of the squirrel monkey (Saimiri sciureus). Folia primatol. 29, 19-42.

Coffey J.C. & Johnsonbaugh R.E. (1979) The in vitro metabolism of progesterone, 4-androstene-3,17-dione, testosterone and 17 beta-hydroxy-5 alpha-androstan-3-one by fetal rhesus monkey testes. Steroids 33, 427-434.

Collins D.C., Graham C.E. & Preedy J.R. (1975) Indentification and measurement of urinary estrodiol-17 beta, estriol, pregnanediol and androsterone during the menstrual cycle of the orangutan. Endocrinology 96 93-101.

Corker C.S. & Davidson D.W. (1978) A radioimmunoassay for testosterone in various biological fluids without chromatography. J. Steroid Biochem. 9, 373-374.

Corker C.S., Michie E., Hobson R. & Parboosingh J. (1976) Hormonal patterns in conceptual cycles and early pregnancy. Br. J. Obstet. Gynaecol. 83, 489-494.

Cospinschi G., L'Hermite M., Laclerco R., Golstein J., Vanhaelst L., Virasoro E. & Robyn C. (1975) Effects of glucocorticoids on pituitary hormonal responses to hypoglycaemia. Inhibition of prolactin release. J. Clin. Endocrinol. Metab. 40, 442-449.

Crisp T.M. Dessouky D.A. & Denys F.R. (1973) The fine structure of the human corpus luteum of term pregnancy. Am. J. Obstet. Gynecol. 115, 901-911.

Csapo A.I. (1956) Progesterone 'block'. Am. J. Anat. 98, 273-291.

Csapo A.I. (1959) Studies on excitation-contraction coupling. Ann. N.Y. Acad. Sci. 81, 453-467.

Csapo A.I. (1977) The 'sea-saw' theory of parturition in, The Fetus and Birth, Ciba Found. Symp. 47, 157-209.

Csapo A.I., Eskola J. & Ruttner Z. (1980) The biological meaning of progesterone levels. Prostaglandins 19, 203-211.

Csapo A.I., Knobil E., vanderMolen H., & Wiest W. (1971) Peripheral plasma levels during pregnancy and labor. Am. J. Obstet. Gynecol. 110, 630-632.

Csapo A.I. & Pulkkinen M. (1978) Indispensability of the human corpus luteum in the maintenance of early pregnancy: luteectomy evidence. Obstet. Gynecol. Surv. 33, 69-89

Csapo A.I., Pulkkinen M.O. & Kaihola H.L. (1973b) The effect of estradiol replacement therapy on early pregnant lutectomized patients. Am. J. Obstet. Gynecol. 117, 987-990.

Csapo A.I., Pulkkinen M.O., Ruttner B., Sauvage J.P. & Wiest W.G. (1972) The significance of the human corpus luteum in pregnancy maintenance. Am. J. Obstet. Gynecol. 112, 1061-1067.

Csapo A.I., Pulkkinen M.O. & Wiest W.G. (1973a) Effects of luteectomy and progesterone replacement therapy in early pregnant patients. Am. J. Obstet. Gynecol. 115, 759-765.

Cutright P. (1975) Spontaneous fetal loss: A note on rates and some implications. J. Biosoc. Sci. 7, 421-433.

Daly M. & Wilson M. (1978) Sex. Evolution and Behaviour Ch. 6 pp. 119-161, Duxbury Press, Massachusetts.

Davies I.J. & Ryan K.J. (1972) Comparative endocrinology of gestation. Vitam. Horm. 30, 223-277.

Davies J., Ryan K.J. & Petro Z. (1970) Estrogen synthesis by adrenal-placental tissues of the sheep and the iris monkey in vitro. Endocrinology 86, 1457-1459.

Dawes G.S. (1968) Foetal and Neonatal Physiology. Year Book Medical Publishers, Chicago.

Dawes G.S. (1976) The physiological determinants of fetal growth. J. Reprod. Fertil. 47, 183-187.

Dawood M.Y. (1977) Hormones in amniotic fluid. Am. J. Obstet. Gynecol. 128, 576-583.

Dawood M.Y. and Fuchs (1980) Estradiol and progesterone in the maternal and fetal circulation in the baboon. Biol. Reprod. 22, 179-184.

Dawood M.Y. & Helmkamp F. (1977) Human umbilical arterial and venous progesterone concentrations: effect of fetal sex, weight and mode of delivery. Obstet. Gynecol. 50, 450-453.

Dawood M.Y. & Saxena B.B. (1977) Testosterone and dihydrotestosterone in maternal and cord blood and in amniotic fluid. Am. J. Obstet. Gynecol. 129, 31-42.

Deane H.W., Lobel B.L. & Romney S.L. (1962) Enzymic histochemistry of normal human ovaries of the menstrual cycle, pregnancy, and the early puerperium. Am. J. Obstet. Gynecol. 83, 281-294.

DeHertogh R., Thomas K., Bietlot Y., Vanderheyden I & Ferin J. (1975) Plasma levels of unconjugated estrone, estradiol and estriol and of HCS throughout pregnancy in normal women. J. Clin. Endocrinol. Metab. 40 93-101.

Dempsey E.W. (1939) The reproductive cycle of New World monkeys. Am. J. Anat. 64, 381-405.

Doe R.P., Fernandez R. & Seal U.S. (1964) Measurement of corticosteroid-binding globulin in man. J. Clin. Endocrinol. Metab. 24, 1029-1039.

Diamond M. (1966) Progestogen inhibition of normal sexual behaviour in the male guinea pig. Nature 209, 1322-1324.

Diczfalusy E. (1953) Chorionic gonadotrophin and oestrogens in the human placenta. Acta. Endocrinol(Copenh) (Suppl) 12 1-175.

Diczfalusy E. (1969) Steroid metabolism in the foeto-placental unit. In The Foeto-placental Unit, pp. 65-109, ed. A. Pecile and C. Finzi, Excerpta Medica Foundation, Amsterdam.

Diczfalusy E. (1974) Endocrine functions of the human fetus and placenta. Am. J. Obstet. Gynecol. 419-433.

Diczfalusy E. & Landgren B-M. (1977) Hormonal changes in the menstrual cycle. In Regulation of Human Fertility, pp. 21-71, ed. E. Diczfalusy, Scriptor, Copenhagen.

Diczfalusy E. & Lindkvist P. (1956) Isolation and estimation of "free" oestrogens in human placenta. Acta Endocrinol, (Copenh) 22, 203-223.

Diczfalusy E. & Troen P. (1961) Endocrine functions of the human placenta. Vitam. Horm. 19, 224-311.

Dierschke D.J., Wehrenberg W.B., Wolf R.C., Clark J.R. & Robinson J.A. (1978) A reversal in the ratio of estrone and estradiol during late pregnancy in the uterine vein of ovariectomized rhesus monkeys. Endocrinology 103, 486-491.

Diez D'Aux R.C. & Murphy B.E.P. (1974) Androgens in the human fetus. J. Steroid. Biochem. 5, 207-210.

Dighe K.K. & Hunter W.M. (1974) A solid-phase radioimmunoassay for plasma progesterone. Biochem. J. 143, 219-231.

Dorfman R.I. (1967) The antiestrogenic and antiandrogenic activities of progesterone in the defence of a normal fetus. Anat. Rev. 157, 547-557.

Dörner G., Stahl F. & Baumgarten G. (1972) Signifikante Unterschiede im Testosteron und 11-OHCS-Gehalt des Fruchtwassers zwischen männlichen und weiblichen Föten. Endokrinologie 60, 285-288.

Duchen M.R. & McNeilly A.S. (1980) Hyperprolactinaemia and long-term lactational amenorrhoea. Clin. Endocrinol (Oxf) 12, 621-627.

Effer S.B., Gupta M., Younglai E.V. (1973) Concentrations of human chorionic gonadotropin, progesterone, and unconjugated and total estriol in umbilical artery and vein plasma at term. Am. J. Obstet. Gynecol. 116, 643-647.

Ehara Y., Yen S.S.C. & Siler T.M. (1975) Serum prolactin levels during puberty. Am. J. Obstet. Gynecol. 121, 995-999

Elliott M.E., Sehgal P.K. & Chalifoux L.V. (1976) Managment and breeding of Aotus trivirgatus. Lab. Anim. Sci. 26, 1037-1040.

Epple G. (1967) Vergleichende Untersuchungen uber Sexual und Sozialverhalten der Krallenaffen (Hapalidae). Folia primatol. 7, 37-65.

Erickson C.J., Bruder R.H., Komisaruk B.R. & Lehrman D.S. (1967) Selective inhibition by progesterone of androgen-induced behaviour in male ring doves (Streptopelia risoria). Endocrinology 81, 39-44.

Erpino M.J. & Chappelle T.C. (1971) Interactions between androgens and progesterone in mediation of aggression in the mouse. Horm. Behav. 2, 265-272.

Escarcena L., Clark H. & Gurpide E. (1978) Contribution of maternal circulation to blood-borne progesterone in the fetus. I. Studies on human subjects. Am. J. Obstet. Gynecol. 130, 462-465.

Fogel M., Rubin B.L. & Ossowski R. (1972) Serum follicle-stimulating and luteinizing hormone and progesterone in single and multiple gestations following induction of ovulation. Am. J. Obstet. Gynecol. 112, 629-639.

Forest M.G., Ances I.G., Tapper A.J. & Migeon C.J. (1971) Percentage binding of testosterone, androstenedione and dehydroisoandroterone in plasma at the time of delivery. J. Clin. Endocrinol. Metab. 32, 417-425.

Forest M.G., Cathiard A.M. & Bertrand J.A. (1973) Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: Use of a sensitive radioimmunoassay for testosterone. J. Clin. Endocrinol. Metab. 36, 1132-1142.

Frandsen V.A. & Stakemann G. (1961) The site of production of oestrogenic hormones in human pregnancy. Acta Endocrinol. (Copenh) 38, 383-390.

Froewis J. (1963) When does adequate reversal of production of the pregnancy-maintaining hormone of the pregnancy

corpus luteum for the placenta occur? A clinical contribution. Wien. Klin. Wschr 75, 368-370.

Fuchs F. (1977) Endocrinology of labor in Endocrinology of Pregnancy pp. 327-349, eds. F. Fuchs and A. Klopper, Harper and Row, London.

Furuyama S., Mayes D.M. and Nugent C.A. (1970) A radioimmunoassay for plasma testosterone. Steroids 16, 415-428.

Fylling P. (1970) The effect of pregnancy, ovariectomy and parturition on plasma progesterone level in the sheep. Acta Endocrinol (Copenh). 65, 273-283.

Fylling P. (1970b) Disappearance rate on progesterone following simultaneous removal of the corpus luteum and the foeto-placental unit in women. Acta Endocrinol. (Copenh). 65, 284-292.

Gandy H.M. (1971) Androgens in Endrocrinology of Pregnancy. pp. 101-154, eds. F. Fuchs and A. Klopper, Harper and Row, London.

Gandy H.M. (1977) Androgens in Endocrinology of Pregnancy. pp. 123-156, eds. F. Fuchs and A. Klopper, Harper and Row, London.

Garner P.R. & Armstrong D.T. (1977) The effect of human chorionic gonadotropin and estradiol-17β on the maintenance of the human corpus luteum of early pregnancy. Am. J. Obstet. Gynecol. 128, 469-475.

Geiger W., Kaiser R. & Franchimont P. (1971) Comparative radioimmunological determination of human chorionic gonadotrophin, human placental lactogen, growth hormone and thyrotrophin in foetal and maternal blood after delivery. Acta Endocrinol. (Copenh) 68, 169-182.

Gengozian N. (1971) Male and female cell populations in the chimeric marmoset; in Medical primatology 1970. Proc. 2nd Conf. Exp. Med. Surg. in Primates, New York 1969, pp. 926-938, eds. Goldsmith and Moor-Jankowski, Karger, Basel.

Gengozian N., Batson J.S., Greene C.T. & Gosslee D.G. (1969) Hemopoietic chimerism in imported and laboratory-bred marmoset. Transplantation 8, 633-652.

Gengozian N., Batson J.S. & Eide P. (1964) Hematologic and cytogenetic evidence for chimerism in the marmoset, Tamarinus nigricollis. Cytogenet. 3, 384-393.

Gengozian N. & Dienhardt F. (1978) eds. Primates in Medicine, vol. 10. Marmoset in experimental Medicine. Karger, Basle.

Gengozian N. & Patton M.L. (1972) Identification of three blood factors in the marmoset, Saguinus fuscicollis ssp,; Medical primatology 1972, Proc. 3rd Conf. Exp. Med. Surg. in Primates. Lyon 1972, part 1, pp. 349-360, eds. Goldsmith and Moor-Jankowski. Karger, Basel.

Gibbons J.M., Mitnick M. & Chieffo V. (1975) In vitro biosynthesis of TSH- and LH- releasing factors by the human placenta. Am. J. Obstet Gynecol. 121, 127-131.

Giles H.R., Lox C.D., Heine M.W. & Christian C.C. (1974) Intrauterine fetal sex determination by radioimmunoassay of amniotic fluid testosterone. Gynecol. Invest. 5, 317-323.

Goebelsmann U. (1979) Protein and steroid hormones in pregnancy. J. of Reprod. Med. 23, 166-177.

Golander, A., Barrett J.R., Tyrey, L, Fletcher W.H. & Handwerger S. (1978) Differential synthesis of human placental lactogen and human chorionic gonadotropin in vitro. Endocrinolgoy 102, 597-605.

Goncharov N., Aso T., Cekan Z., Pachalia E. & Diczfalusy E. (1976) Hormonal changes during the menstrual cycle of the baboon (Papio hamadryas). Acta Endocrinol. (Copenh) 82, 396-412.

Gondos B. & Hobel C.J. (1971) Ultrastructure of germ cell development in the human fetal tesis. Z. Zellforsch. 119, 1-20

Goodman A.L., Nixon W.E., Johnson D.K. & Hodgen G.D. (1977) Regulation of folliculogenesis in the cycling rhesus monkey: selection of the dominant follicle. Endocrinology 100, 479-481

Gordon Y.B. & Chard T. (1979) The specific proteins of the human placenta - some new hypotheses. In Placental Proteins pp 1-22, eds: A. Klopper & T. Chard, Springer-Verlag, Berlin.

Goss C.M., Popejoy L.T. Fusiler J.L. & Smith T.M. (1968) Observations on the relationship between embryological development, time of conception and gestation in, The Squirrel Monkey, pp. 171-191, eds. L.A. Rosenblum and R.W. Cooper, Academic Press, New York.

Goy R.W., Wolf J.E. & Eisele S.G. (1977) Experimental female hermaphroditism in rhesus monkeys: anatomical and psychological characteristics, in Handbook of Sexology, pp. 140-156, eds. J. Money, and H. Musaph. Elsevier/North Holland Biomedical Press, Amsterdam.

Green J.A., Garcilcilazo J.A. & Maqueo M. (1967) Ultrastructure of the human ovary. II. The luteal cell at term. Am. J. Obstet. Gynecol. 99, 855-863.

Greenwood F.C., Hunter W.M. & Glover J.S. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. 89, 114-123.

Gruenwald P. (1966) Growth of the human fetus. Am. J. Obstet. Gynecol. 94, 1112-1119.

Gruenwald P. & Minh H.N. (1960) Evaluation of body and organ weights in perinatal pathology: I. Normal standards derived from autopsies. Am. J. Clin. Pathol. 34, 247-253.

Gulyas B.J. (1974) The corpus luteum of the rhesus monkey (Macaca mulatta) during late pregnancy. An electron microscopic study. Am. J. Anat. 139, 95-121.

Guraya S.S. (1972) Function of the human ovary during pregnancy as revealed by histochemical, biochemical and electron microscope techniques. Acta. Endocrinol. (Copenh) 69, 107-118.

Hagemenas F.C. & Kittinger G.W. (1972) The influence of fetal sex on plasma progesterone levels. Endocrinology 91, 253-256.

Hagemenas F.C. & Kittinger G.W. (1973) The influence of fetal sex on the level of plasma progesterone in the human fetus. J. Clin Endocrinol. Metab. 36, 398-391.

Hagemenas F.C. & Kittinger G.W. (1974) The effect of fetal sex on placental biosynthesis of progesterone. Endocrinology 94, 922-924.

Hagemenas F.C., Baughman W.L. & Kittinger G.W. (1975) The effect of fetal hypophysectomy on placental biosynthesis of progesterone in rhesus monkeys. Endocrinology 96, 1059-1062.

Halme J., Ikonen M., Rutanen E.M. & Seppala M. (1978) Gonadotropin receptors of human corpus luteum during the menstrual cycle and pregnancy. Am. J. Obstet. Gynecol. 131, 728-734.

Hammerstein J., Rice B.F. & Savard K. (1964) Steroid hormone formation in the human ovary. I. Identification of steroids formed in vitro from acetate-1-14C in the corpus luteum. J. Clin. Endocrinol. Metab. 24 597-605.

Hampton S.H. (1975) Placental development in the marmoset in, Contempory Primatology, pp. 106-114, eds. S. Kondo, M. Kawai and A. Ehara, Karger, Basel.

Hampton S.H., Gross M.J. & Hampton J.K. (1978) A comparison of captive breeding performance and offspring survival in the family Callitricidae, in, Marmosets in Experimental Medicine pp. 88-95, ed. N. Gengozian and F. W. Dienhardt, Primates in Medicine, vol. 10, Karger, Basle.

Hampton J.K. jr. & Hampton S.H. (1965) Marmosets (Hapalidae): Breeding seasons, twinning and sex of offspring. Science N.Y. 150, 915-917.

Hampton J.K. & Hampton S.H. (1975) Some expected and unexpected characteristics of reproduction in Callithricidae. In, Breeding Simians for Developmental Biology (Lab. Anim. Handbook No.6) pp. 235-240, eds. F. Perkins and P. O'Donoghue. Laboratory Animals Ltd., London.

Hampton S.H. & Taylor A.C. (1971) Gonadal development in marmosets. Proc. 3rd Int. Congr. Primat Zurich, Vol. 1 pp. 246-259. Karger, Basel.

Hanson F.W., Powell J.E. & Stevens V.C. (1971) Effect of hCG and hLH on steroid secretion and functional life of corpus luteum. J. Clin. Endocrinol. Metab. 32, 211-215.

Harcourt A., Fossey D., Stewart K.J. & Watts D.P. (1980) Reproduction in wild gorillas and some comparisons with chimpanzees. J. Reprod. Fertil. (suppl). 28, 59-70.

Harrison R.F., Youssefnejadian E., Brodovcky H., Johnson M. & Dewhurst J. (1978) Secretion patterns of plasma progesterone, 17-hydroxyprogesterone and 20α-hydroxypregn-4-en-3-one in early normal pregnancy. Br. J. Obstet. Gynaecol. 85, 921-926.

Heap R.B. & Illingworth D.V. (1974) The maintenance of gestation in the guinea-pig and other hystricomorph rodents; changes in the dynamics of progesterone metabolism and the occurrence of progesterone-binding globulin (PBG). Symp. Zool. Soc. Lond. 34, 385-415.

Heap R.B. & Perry J.S. (1974) The maternal recognition of pregnancy. Br. J. Hosp. Med. July 8-14.

Heap R.B., Perry J.S. & Challis J.R. (1973) Hormonal maintenance of pregnancy, in, Handbook Physiol. Sect. 7 Endocrinology, Female Reproductive System, Part 2. pp. 217-260, eds. R.O. Greep and E.B. Astwood. American Physiological Society, Washington D.C.

Hearn J.P. (1976) Immunisation against pregnancy. Proc. R. Soc. Lond. (Biol.) 195, 149-160.

Hearn J.P. (1977) Restraining device for small monkeys. Lab. Anim. 11, 261-262.

Hearn J.P. (1978) The endocrinology of reproduction in the common marmoset, Callithrix jacchus. In, The Biology

and Conservation of Marmosets, pp. 163-171, ed. D.G. Kleiman. Smithsonian Institute, Washington, D.C.

Hearn J.P., Abbott D.H., Chambers P.L., Hodges J.K. and Lunn S.F. (1978) Use of the common marmoset, Callithrix jacchus, in reproductive research, in, Marmosets in Experimental Medicine, ed. N. Gengozian and F.W. Dienhardt Primates in medicine, vol. 10, pp. 40-49, Karger, Basle.

Hearn J.P. & Lunn S.F. (1975) The reproductive biology of the marmoset monkey, Callithrix jacchus. In, The Breeding of Simians and Their Uses in Development Biology (Lab. Anim. Handbook No.6), pp. 191-202, eds. F.T. Perkins and P.N. O'Donoghue, Laboratory Animals Ltd. London.

Hearn J.P., Lunn S.F., Burden F.J. & Pilcher M.M. (1975) Management of marmosets for biomedical research. Lab. Anim. 9, 125-134.

Hearn J.P. & Renfree M.B. (1975) Prealbumins in the vaginal flushings of the marmoset, Callithrix jacchus. J. Reprod. Fert. 43, 159-161.

Hearn J.P., Short R.V. & Lunn S.F. (1975) The effect of immunising marmoset monkeys against the β-subunit of HCG. In, Physiological Effects of Immunity against Reproductive Hormones, pp. 229-247, eds. R.G. Edwards and M.H. Johnson. University Press, Cambridge.

Heinrichs W.L. & Colas A.E (1970) Hepatic microsomal 16-alpha-hydroxylation of 3-beta-hydroxyandrost-5-en-17-one (DHA) by fetal, newborn, and adult rhesus monkeys. Gen. Comp. Endocrinol 14, 159-163.

Hendricks C.H. (1964) Patterns of fetal and placental growth:

the second half of normal pregnancy. Obstet Gynecol 24, 357-365.

Hendrickx A.G. (1971) Embryology of the Baboon, Univ. of Chicago Press, Chicago.

Hendrickx A.G. (1972) Early development of the embryo in non-human primates and man. Acta Endocrinol (suppl) (Copenh) 166, 103-130.

Hendrickx A.G., Sawyer R.H., Laslay B.L. and Barnes R.D. (1975) Comparison of development stages in primates with a note on the detection of ovulation. In, Breeding Simians for Developmental Biology (Lab. Anim. Handbook No.6), pp. 305-315, ed. F. Perkins and P.N. O'Donoghue. Laboratory Animals Ltd., London.

Hess D.L., Hendrickx A.G. & Stabenfeldt G.H. (1979) Reproductive and hormonal pattern in the African Green monkey (Cercopithecus aethiops). J. Med. Primatol. 8, 273-281.

Heuser C.H. & Streeter G.L. (1941) Development of the macaque embryo. Contrib. to Embryol. 29, 15-55.

Hill J.P. (1926) Development of Hapale jacchus. J. Anat. (Lond.) 60, 486-487.

Hill J.P. (1932) The developmental history of the primates. Croonian Lecture. Phil. Trans. 221, 45-178.

Hill J.P. & Hill C.J. (1927) An early blastocyst of Hapale. J. Anat. 61, 514.

Hindson J.C., Scho field B.M. & Ward W.R. (1969) The effect of progesterone on recorded parturition and on oxytocin sensitivity in the sheep. J. Endocrinol. 43, 207-215.

Hobson B.M. (1971) Production of gonadotrophin, oestrogens and progesterone by the primate placenta; in Bishop, Advances in Reproductive Physiology, vol. 5, pp. 67-102, Academic Press, New York.

Hobson B.M. (1972) Gonadotrophin concentrations in the placentae of man, the rhesus monkey and the marmoset. Folia primatol. 18, 35-40.

Hobson B.M., Hearn J.P., Lunn S.F. & Flockhart J.H. (1977) Urinary excretion of biologically active chorionic gonadotrophin by the pregnant marmoset (Callithrix jacchus jacchus). Folia primatol. 28, 251-258.

Hobson B.M. & Wi de L. (1972) Comparison between chorionic gonadotrophins extracted from human, rhesus monkey and marmoset placentae. J. Endocrinol. 55 363-368

Hobson B. & Wide L. (1974) Chorionic gonadotrophin in the human placenta in relation to the sex of the foetus at term. J. Endocrinol. 60 75-80.

Hobson B.M. & Wide L. (1981) The similarity of chorionic gonadotrophin and its subunits in term placentae from man apes, Old and New World monkeys and a Prosimian. Folia primatol. (in press).

Hobson W., Coulston F., Raiman C., Winter J.S.D. & Reyes F. (1976) Reproductive endocrinology of female chimpanzees. A suitable model of humans. J. Toxicol. Environ. Health 1, 657-662.

Hodgen G.D., Dufau M.L., Catt K.J. & Tullner W.W. (1972) Estrogens, progesterone and chorionic gonadotropin in pregnant rhesus monkeys. Endocrinology 91, 896-900.

Hodgen G.D., Stouffer R.L., Barber D.L. & Nixon E. (1977) Serum estradiol and progesterone during pregnancy and the status of the corpus luteum at delivery in cynomolgus monkeys (Macaca fascicularis). Steroids 30, 295-301.

Hodgen G.D. & Tullner W.W., (1974) Plasma estrogens and progesterone in pregnant rhesus monkeys (Macaca mulatta) after fetectomy or ovariectomy. Endocrinology 94 (suppl) A248.

Hodgen G.D. & Tullner W.W. (1975) Plasma estrogens, progesterone and chorionic gonadotropin in pregnant rhesus monkeys (Macaca mulatta) after ovariectomy. Steroids, 25, 275-282.

Hodgen G.D., Tullner W.W., Vaitukaitis J., Ward D. & Ross G.T. (1974) Specific radioimmunoassay of chorionic gonadotropin during implantation in rhesus monkeys. J. Clin. Endocrinol. Metab. 39, 457-463.

Hodgen G.D., Wolfe L.C., Ogden J.O., Adams M.R., Descalzi C.C. & Hildebrand D.F. (1976) Diagnosis of pregnancy in marmosets: Hemagglutination inhibition test and radioimmunoassay for urinary hCG. Lab. Anim. Sci. 26, 224-229.

Hodges J.K. (1977) The control of luteinising hormone secretion in the marmoset monkey, Callithrix jacchus. Ph.D. thesis dissertation, University of Edinburgh.

Hodges J.K. (1978) Effects of gonadectomy and oestradiol treatment on plasma luteinizing hormone concentrations in the marmoset monkey, Callithrix jacchus. J. Endocrinol. 76, 271-281.

Hodges J.K. & Hearn J.P. (1978) Long term suppression of fertility by immunisation against LHRH and its reversibility

in female and male marmoset monkeys. In, Recent Advances in Reproduction and Regulation of Fertility. pp. 87-96, ed. G.P. Talwar. Elsevier/North-Holland Biomedical Press. Amsterdam.

Holmdahl T.H., Johansson E.D.B. & Wide L. (1971) The site of progesterone production in early pregnancy. Acta Endocrinol. (Copenh) 67, 353-361.

Hopf S. (1967) Notes on pregnancy, delivery, and infant survival in captive squirrel monkeys. Primates 8, 323-332.

Horton R. & Tait J.F. (1966) Androstenedione production in peripheral blood and studies on the possible sites of its conversion to testsoterone. J. Clin. Invest. 45, 301-313.

Hoschoian J. C. & Brownie A.C. (1967) Pathways for androgen biosynthesis in monkey testis. Steroids 10, 49-69.

Hotchkiss J., Atkinson L.E. & Knobil E. (1971) Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey. Endocrinology 89, 177-183.

Howie P.W. & McNeilly A.S. (1979) Lactational amenorrhoea prolactin and contraception. Eur. J. Clin. Invest. 9, 237-238.

Huggett A.St. G. & Widdas W.F. (1951) The relationship between mammalian foetal weight and conception age. J. Physiol. 114, 306-317.

Huhtaniemi I.T., Korenbrot C.C. & Jaffe R.B. (1977) hCG binding and stimulation of testosterone biosynthesis in the human fetal testes. J. Clin. Endocrinol. Metab. 44, 963-967.

Huot R.I., Foidart J.-M. & Stromberg K. (1979) Effects of culture conditions on the synthesis of human chorionic gonadotropin by placental organ cultures, In Vitro 15, 497-502.

Hussa R.O., Patillo R.A., Ruckert A.C. & Scheuermann K.W. (1978) Effects of butyrate and dibutyryl cyclic AMP on hCG-secreting trophoblastic and non-trophoblastic cells. J. Clin. Endocrinol. Metab. 46, 69-76.

Hussa R.O., Story M.T. & Patillo R.A. (1974) Cyclic adenosine monophosphate stimulates secretion of human chorionic gonadotropin and estrogens by human trophoblast in vitro. J. Clin. Endocrinol. Metab. 38, 338-340.

Illingworth D.V., Heap R.B. & Perry J.S. (1970) Changes in the metabolic clearance rate of progesterone in the guinea pig. J. Endocrinol. 48 409-413.

Ingram J.C. (1975) Husbandry and observation methods of a breeding colony of marmoset (Callithrix jacchus) for behavioural research. Lab. Anim. 9, 249-259.

Ingram J.C. (1977) Interactions between parents and infants, and the development of independence in the common marmoset, Callithrix jacchus. Anim. Behav. 25, 811-827.

Jacobi J., Lloyd H.M. & Meares J.D. (1977) Onset of oestrogen induced prolactin secretion and DNA synthesis by the rat pituitary gland. J. Endocrinol. 72 35-40.

James W.H. (1970) The incidence of spontaneous abortion. Popul. Stud. 24, 241-242.

Jirku H. & Layne D.S. (1965) The metabolism of estrone-^{14}C in a pregnant chimpanzee. Steroids 5, 37-44.

Johannson E.D.B. (1969) Plasma levels of progesterone in

pregnancy measured by a rapid competitive protein binding technique. Acta Endocrinol (Copenh) 61, 607-614.

Johansson E.D.B. & Jonasson L.E. (1971) Progesterone levels in amniotic fluid and plasma from women. Acta Obstet. Gynecol. Scand. 50, 339-343.

Jost A. (1947) Sur le role des gonades foetales dans la differenciation sexuelle somatique de l'embryon de lapin. C.R. Assoc. Anat. 34 255-262.

Jost A. (1970a) General outline about reproductive physiology and its developmental background. 21 Colloquium der Gesellshaft fur Biologische Chemie. 1-32. Mosbach/Baden. Springen-Verlag, Berlin.

Jost A. (1970b) Hormonal factors in the sex differentiation of the mammalian fetus. Philos. Trans. R. Soc. Lond. (Biol.) 259, 119-131.

Jovanovic L., Dawood M.Y., Landesman R. & Saxena B.B. (1978) Hormonal profile as a prognostic index of early threatened abortion. Am. J. Obstet Gynecol. 130, 274-278.

Kalla N.R., Nisula B.C., Menard R. & Loriaux D.L. (1980) The effect of estradiol on testicular testosterone biosynthesis (rat). Endocrinology 106, 35-39.

Kaplan S.L. & Grumbach M.M. (1978) Pituitary and placental gonadotrophins and sex steroids in the human and subhuman primate fetus. Clin. Endocrinol. Metab. 7, 487-511.

Karow W.G. & Gentry W.C. (1976) Corpus luteum function during pregnancies of previously infertile women. Obstet. Gynecol. 48, 603-606.

Karim S., Rao B. & Salmon J. (in press; referred to in Rao, 1979). Changes in plasma hormone levels after corpus luteum removal and following surgical abortion in early pregnancy.

Katzenellenbogen B.S. & Ferguson E.R. (1975) Antiestrogen action in the uterus: Biological ineffectiveness of nuclear bound estradiol after antiestrogen. Endocrinology 97, 1-8.

Kauppila A., Jouppila P., Karvonen P., Tuimala R. and Ylikorkala O. (1976) Effect of dexamethasone on blood levels of ACTH, cortisol, progesterone, estradiol and estriol during late pregnancy. Int. J. Gynecol. Obstet. 14, 177-184.

Kauppila A., Puukka M. & Tuimala R. (1979) Effect of dexamethasone on prolactin secretion in late pregnancy. Am. J. Obstet. Gynecol. 134, 752-755.

Kelly R.W. (1971) The measurement by gas chromatography-mass spectrometry of oestra-1,3,5-triene-3,15,16,17β-tetrol (oest etrol) in pregnancy urine. J. Chromatogr. 54, 345-355.

Kelly R.W. & Taylor P.L. (1976) Tert-butyl dimethyl-silyl ethers as derivatives for qualitative analysis of steroids and prostaglandins by gas phase methods. Anal. Chem. 48, 465-467.

Kenny F.M., Angsusingha, Stinson D. & Hotchkiss J. (1973) Unconjugated estrogens in the perinatal period. Pediatr. Res. 7, 826-831.

Kerr G.R., Allen J.R., Scheffler G. & Couture J. (1974) Fetal and postnatal growth of rhesus monkeys (M. mulatta). J. Med. Primatol. 3, 221-235.

Kerr G.R., Kennan A.P., Waisman H.A. & Allen J.R. (1969) Growth and development of the fetal rhesus monkey. I. Physical growth. Growth 33, 201-213.

Kerr G.R. & Waisman H.A. (1971) Fetal biology of the rhesus monkey (Macaca mulatta), in Medical primatology 1970. Proc. 2nd Conf. Exp. Med. Surg. Primates. New York 1969, pp. 590-602. Karger, Basel.

Khodr G. & Siler-Khodr T.M. (1978) The effect of luteinizing hormone-releasing factor on human chorionic gonadotropin secretion. Fertil Steril. 30, 301-304.

Kim M.H., Borth R., McCleary P.H., Woolever, A & Young, P. (1971) Sex hormone secretion of the placenta left in situ after ovarian pregnancy. Am. J. Obstet. Gynecol. 110, 658-662.

Kleiman D.G. (1977) (ed). The Biology and Conservation of the Callitrichidae, Smithsonian Institute Press, Washington.

Kletzky O.A., Marrs R.P., Howard W.F., McCormick W. & Mishell D.R. (1980) Prolactin synthesis and release during pregnancy and puerperium. Am. J. Obstet. Gynecol. 136, 545-550.

King O.R. & Westfahl P.K. (1978) Steroid changes during the menstrual cycle of the baboon (Papio cycnocephalus) and human. Biol. Reprod. 18, 392-400.

Klinga K., Bek E. & Runnebaum B. (1978) Maternal peripheral testosterone levels during the first half of pregnancy. Am. J. Obstet. Gynecol. 131 60-62.

Klopper A. (1970) Steroids in amniotic fluid. Ann. Clin. Res. 2, 289-299.

Klopper A. (1977) Choice of hormone assay in the assessment of feto-placental function. In, Endocrinology of Pregnancy, pp. 350-364, eds. F. Fuchs and A. Klopper,

Harper and Row, London.

Klopper A. & Fuchs F. (1977) Progestagens. In, Endocrinology of Pregnancy, pp. 123-156, eds. F. Fuchs and A. Klopper, Harper and Row, New York.

Knobil E. (1973) On the regulation of the primate corpus luteum. Biol. Reprod. 8, 246-258.

Koering M.J., Wolf R.C. & Meyer R.K. (1973) Morphological and functional evidence for corpus luteum activity during late pregnancy in the rhesus monkey. Endocrinology 93, 686-693.

Kosasa T., Levesque L., Goldstein D.P. & Taymor M.L. (1973) Early detection of implantation using a radioimmunoassay specific for human chorionic gonadotropin. J. Clin. Endocrinol. Metab. 36, 622-624.

Kunz J. & Keller P.J. (1976) HCG, HPL, oestradiol, progesterone and AFP in serum in patients with threatened abortion. Br. J. Obstet. Gynaecol. 83, 640-644.

Lamb E., Mancuso S., Dell'Acqua S., Wiqvist N. & Diczfalusy E. (1967) Studies on the metabolism of C-19 steroids in the human foeto-placental unit. Acta Endocrinol. (Copenh) 55, 263-277.

Lanman J.T. (1957) The adrenal fetal zone, its occurrence in primates and a possible relationship to chorionic gonadotropin. Endocrinology 61, 684-691.

Lanman J.T. (1977) Parturition in non-human primates. Biol. Reprod. 16, 28-38.

Lanman J.T., Thau R., Sundaram K., Brinson A. & Bonk R. (1975) Ovarian and placental origins of plasma progesterone following fetectomy in monkeys (Macaca mulatta).

Endocrinology 96, 591-597.

Lees M.H., Hill J.D., Ochsner A.J., Thomas C.L. & Novy M.J. (1971) Maternal placental and myometrial blood flow of the rhesus monkey during uterine contractions. Am. J. Obstet. Gynecol. 110, 68-81.

Leitch I., Hytten F.E. & Billewicz W.Z. (1959) The maternal and neonatal weights of some mammalia. Proc. Zool. Soc. Lond. 133, 11-28.

LeMaire W.J., Conly P., Moffett A. & Cleveland W. (1970) Plasma progesterone severtion by the corpus luteum of term pregnancy. Am. J. Obstet. Gynecol. 108, 132-134.

LeMaire W.J., Rice B.F. & Savard K. (1968) Steroid hormone formation in the human ovary: V. Synthesis of progesterone in vitro in corpora lutea during the reproductive cycle. J. Clin. Endocrinol. Metab. 28, 1249-1256.

Leung K. & Solomon S. (1972) Metabolism of progesterone in the fetus and placenta of the rhesus monkey (Macaca mulatta). Endocrinology 91, 341-349.

Leutenegger W. (1970a) Das Becken der rezenten Primaten. Morphol. Jb. 115, 1-101.

Leutenegger W. (1970b) Beziehungen zwischen der Neugeborenengrösse und dem Sexual-dimorphismus am Becken bei simischen Primaten. Folia Primatol. 12, 224-235.

Leutenegger W. (1973) Maternal-fetal relationships in primates. Folia Primatol 20, 280-293.

Levitz M. & Young B.K. (1977) Estrogens in pregnancy. Vitam. Horm. 35, 109-147.

Liggins G.C., Fairclough R.J., Grieves S.A., Kendall J.Z.

& Knox B.S. (1973) The mechanism of initiation of parturition in the ewe. Recent Prog. Horm. Res. 29, 111-118.

Liggins G., Forster C., Grieves S. & Schwartz A. (1977). Control of parturition in man. Biol. Reprod. 16 39-56.

Lin T.J., Lin S.T., Erlenmeyer F., Kline I.T., Underwood R., Billiar R.B. & Little B. (1972) Progesterone production rates during the third trimester of pregnancy in normal women, diabetic women and women with abnormal glucose tolerance. J. Clin. Endocrinol. Metab. 34, 287-297.

Lindberg B.S., Johansson E.D.B. & Nilsson B.A. (1974) Plasma levels of non-conjugated oestrone, oestradiol-17β and oestriol during uncomplicated pregnancy. Acta. Obstet. Gynecol. Scand. (suppl) 32, 21-26.

Lindberg B.S., Nilsson B.A. & Johansson E.D. (1974) Plasma progesterone levels in normal and abnormal pregnancy. Acta Obstet. Gynecol. Scand. 24, 73-80.

Linzell J.L. & Heap R.B. (1968) A comparison of progesterone metabolism in the pregnant sheep and goat: sources of production and an estimation of uptake by some target organs. J. Endocrinol. 41, 433-439.

Liskowski L. and Wolf R.C. (1972) Urinary excretion of progesterone metabolites in pregnant rhesus monkeys. Proc. Soc. Exp. Biol. Med. 139, 1123-1126.

Liskowski L., Wolf R.C., Chandler S. & Meyer R.K. (1970) Urinary estrogen excretion in pregnant rhesus monkeys. Biol. Reprod. 3 55-60.

Longcope C., Layne D.S. & Tait J.F. (1968) Metabolic clear ance rates and interconversions of estrone and 17-beta-

estradiol in normal males and females. J. Clin. Invest. 47, 93-106.

Longcope C., Kato T. & Horton R. (1969) Conversion of blood androgens to estrogens in normal adult men and women. J. Clin. Invest. 48, 2191-2201.

Lunn S.F. (1978) Urinary oestrogen excretion in the common marmoset, Callithrix jacchus. In, Biology and Behaviour of Marmosets, pp. 67-73, eds. H. Rothe, H.-J. Wolters and J.P. Hearn. Proc. Marmoset Workshop, Gottingen, 1977. Gottingen: Eigenverlag Rothe.

Lunn S.F. (1981) The reproductive biology of the common marmoset, Callithrix jacchus. Ph.D. dissertation, Edinburgh University.

Lunn S.F. and Hearn J.P. (1978) Breeding marmosets for medical research. In, Recent Advances in Primatology. Vol. 2 - "Conservation", pp. 183-185, eds. D.J. Chivers and W. Lane Petter. Academic Press, London.

Lunn S.F. & McNeilly A.S. (1981) Failure of lactation to have a consistent effect on interbirth interval in the common marmoset, Callithrix jacchus jacchus. Folia Primatol. (in press).

Lurie A.O., Reid D.E. & Villee C.A. (1966) The role of the fetus and placenta in maintenance of plasma progesterone. Am. J. Obstet. Gynecol. 96, 670-675.

McCarthy M.S., Walsh S.W. & Novy M.J. (1980) Selective placental secretion of estrogens into fetal and maternal circulation in rhesus monkeys. (Abstract) Progr. End. Soc. 62nd. Meet. 1980.

McDonald G.J., Yoshinga K. & Greep R.O. (1973) Progesterone values in monkeys near term. Am. J. Phys. Anthropol. 28, 201-205.

McDonald P.C., Rombaut R.P. & Siiteri P.K. (1967) Plasma precursors of estrogen. I. Extent of conversion of plasma delta-4-androstenedione to estrone in normal males and non-pregnant, normal, castrate and adrenalectomized females. J. Clin. Endocrinol. Metab. 27, 1103-1111.

McKeown J. & Record R.G. (1953) The influence of placental size on foetal growth in man, with special reference to multiple pregnancy. J. Endocrinol. 9, 418-426.

McKeown T., Marshall T. & Record R.G. (1976) Influences on fetal growth. J. Reprod. Fertil. 47, 167-181.

McKeown T. & Record R.G. (1952) Observations on foetal growth in multiple pregnancy in man. J. Endocrinol. 8, 386-401.

McKim P., Hutchinson T.C. & Gavan J.A. (1972) Prenatal growth of long bones in rhesus and squirrel monkeys (Macaca mulatta and Saimiri sciureus). Am. J. Phys. Anthropol. 36, 353-358.

McNatty K.P., Baird D.T., Bolton A., Chambers P., Corker C. & McLean H. (1976) Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J. Endocrinol. 71, 77-85.

McNatty K.P., Henderson K.M. & Sawers R.S. (1975) Effects of prostaglandin $F_{2\alpha}$ and E_2 on the production of progesterone by human granulosa cells in tissue culture. J. Endocrinol. 67, 231-240.

McNeilly A.S. (1979) Effects of lactation on fertility. Br. Med. Bull. 35, 151-154.

McNeilly A.S. (1980) Prolactin and the control of gonadotrophin secretion. J. Reprod. Fertil. 58, 537-549.

McNeilly A.S., Abbott D., Lunn S.F., Chambers P.L. and Hearn J.P. (1981) Plasma prolactin concentrations during the ovarian cycle and lactation and their relationship to return of fertility post-partum in the common marmoset (Callithrix jacchus). J. Reprod. Fertil. 62, 353-360.

McNeilly A.S. & Friesen H.G. (1978) Heterologous radioimmunoassay for rabbit prolactin. Endocrinology 102, 1539-1547.

McNeilly A.S., Howle P.W. & Houston M.J. (1981) Relationship between feeding patterns, prolactin and the resumption of ovulation post-partum. In, Research Frontiers in Fertility Regulation, pp. 102-116, eds. G.I. Zatuchni, M. H. Labbok and J. J. Sciarra, Harper and Row, Mexico.

Marshall J.R., Hammond C.B., Ross G.T., Jacobson A., Rayford P. & Odell W.D. (1968) Plasma and urinary chorionic gonadotropin during human pregnancy. Obstet. Gynecol. 32, 760-764.

Matsumoto K., Mahajan D.K. & Samuels L.T. (1974) The influence of progesterone on the conversion of 17-hydroxyprogesterone to testosterone in the mouse testes. Endocrinology 94, 808-814.

Meites J. & Clemens J.A. (1972) Hypothalamic control of prolactin secretion. Vitam. Horm. 30, 165-221.

Meyer R.K. (1972) Chorionic gonadotropin, corpus luteum function and embryo implantation inthe rhesus monkey. In, The Use of Nonhuman Primates in Research on Human Reproduction, pp. 214-217, eds. E. Diczfalusy and C.C. Standley. Karolinska Institutet, Stockholm.

Meyer R.K., Wolf R.C. & Arslan M. (1969) Implantation and maintenance of pregnancy in progesterone-treated ovariectomized monkeys (Macaca mulatta). 2nd Int. Congr. Primatol. 2 30-35.

Migeon C.J., Rivarola M.A. & Forest M.G. (1968) Studies of androgens in transsexual subjects. Effects of oestrogen therapy. Bull. Johns Hopkins Hosp. 123, 128-130.

Mikakawa I., Ikeda I., Nakayama M. & Maeyama M. (1976) Plasma concentrations of cortisol, progesterone and unconjugated oestradiol and oestriol in women with live anencephalic foetuses before, during and after labour. J. Endocrinol. 69, 291-295.

Milhail G. (1970) Hormone secretion by the human ovaries. Gynecol. Invest. 1, 5-20.

Mikhail G. & Allen W.M. (1967) Ovarian function in human pregnancy. Am. J. Obstet. Gynecol. 99, 308-312.

Milevich T. & Axelrod L.R. (1971) Metabolism of 4-14C testosterone by lypophilized baboon placental microsomes. Endocrinology 88, 589-595.

Mishell D.R., Nakamura R.M., Barberia J.M. & Thorneycroft (1974) Initial detection of human chorionic gonadotropin in serum in normal human gestation. Am. J. Obstet. Gynecol. 118, 990-994.

Mishell D.R., Thorneycroft I.H., Nagata Y., Murata T. & Nakamura R.M. (1973) Serum gonadotropin and steroid patterns in early human gestation. Am. J. Obstet. Gynecol. 117, 631-639.

Mitchell S.J. & Jones S.M. (1975) Diagnosis of pregnancy in marmosets (Callithrix jacchus). Lab. Anim. 9, 49-56.

Mizuno M., Lobotsky J., Lloyd C.W., Kobayashi T. & Murasawa Y. (1968) Plasma androstenedione and testosterone during pregnancy and in the newborn. J. Clin. Endocrinol. Metab. 28, 1133-1142.

Mizutani S., Tsujimura T., Akashi S. and Matsumoto (1977) Lack of metabolism of progesterone, testosterone and pregnenolone to 5 alpha-products in monkey and human testes compared with rodent testes. J. Clin. Endocrinol. Metab. 44, 1023-1031.

Nagamani M., McDonough P., Ellegood J.O. & Mahesh V.B. (1979) Maternal and amniotic fluid steroids throughout human pregnancy. Am. J. Obstet. Gynecol. 134, 674-680.

Nagle C.A., Denari J.H., Quiroga S., Riarte A., Merlo A., Germino N.I., Gomez-Argana F. & Rosner J.M. (1979) The plasma pattern of ovarian steroids during the menstrual cycle in capuchin monkeys (Cebus apella). Biol. Reprod. 21, 979-983.

Neill J.D., Johansson E.D.B. & Knobil E. (1967) Levels of progesterone in peripheral plasma during the menstrual cycle of the rhesus monkey. Endocrinology 81, 1161-1164.

Neill J.D., Johansson E.D.B. & Knobil E. (1969) Patterns of circulating progesterone concentrations during the fertile menstrual cycle and the remainder of gestation in the rhesus monkey. Endocrinology 84, 45-48.

Neill J.D. & Knobil E. (1972) On the nature of the initial luteotropic stimulus of pregnancy in the rhesus monkey. Endocrinology 90, 34-38.

Niemi M., Ikonen M. & Hervonen A. (1967) Histochemistry and fine structure of interstitial tissue in human foetal testis. In, Endocrinology of the Testes, Ciba Found. Colloq. Endocrinol. 16, 31-55.

Nishimura H., Takano K., Tanimura T. & Yasuda M. (1968) Normal and abnormal development of human embryos: first report of the analysis of 1,213 intact embryos. Teratology 1, 281-290.

Niswender G.D. & Spies H.G. (1973) Serum levels of luteinizing hormone, follicle-stimulating hormone and progesterone throughout the menstrual cycle of rhesus monkeys. J. Clin. Endocrinol. Metab. 37, 326-328.

Novy M.J. (1977) Endocrine and pharmacological factors which influence the onset of labour in rhesus monkeys. In, The Fetus and Birth, pp. 259-287. Ciba Found. Symp. 47. Excerpta Medica, Elsevier/North-Holland.

Novy M.J., Walsh S.W. & Kittinger G.W. (1977) Experimental fetal anencephaly in the rhesus monkey: effects on gestational length and fetal and maternal plasma steroids. J. Clin. Endocrinol. Metab. 45, 1031-1038.

Ogden J.D., Wolfe L.E. & Deinhardt F.W. (1978) Breeding

Saguinus and Callithrix species of marmosets under laboratory conditions, in, Marmosets in Experimental Medicine pp. 79-83, eds. N. Gengozian and F.W. Dienhardt. Primates in Medicine, Vol. 10, Karger, Basle.

Ohrlander S., Gennser G., Batra S. & Lebech P. (1977) Effect of betamethasone administration on estrone, estradiol-17β, and progesterone in maternal plasma and amniotic fluid. Obstet. & Gynecol. 49, 148-153.

Palmar R., Blair J.A., Eriksson G. & Diczfalusy E. (1966) Metabolism of progesterone and 20α-and 20β-dihydroprogesterone by midterm placentas perfused in situ. Acta Endocrinol (Copenh) 53, 407-414

Payne P.R. & Wheeler E.F. (1967) Growth of the fetus. Nature 215, 849-850.

Pearlman W.H., Crepy O. & Murphy M. (1967) Testosterone-binding levels in the serum of women during the normal menstrual cycle, pregnancy, and the post-partum period. J. Clin. Endocrinol. Metab. 27, 1012-1018.

Pelliniemi L.J. & Niemi M. (1969) Fine structure of the human foetal testes. I. The intestitial tissue. Z. Zellforsch. 99, 507-522.

Penny R., Olambiwonnu N.O. & Frasier S.D. (1976) Measurement of human chorionic gonadotropin (HCG) concentrations in paired maternal and cord sera using an assay specific for the beta subunit of HCG. Pediatrics. 58, 110-116.

Penny R., Parlow A.F. & Frasier S.D. (1979) Testosterone and estradiol concentrations in paired maternal and cord sera and their correlation with the concentration of chorionic gonadotropin. Pediatrics. 64, 604-608.

Phillips I.R. (1975) Macaque and marmoset monkeys as animal models for the study of birth defects. In, The Breeding of Simians and Their Uses in Development Biology (Lab. Anim. Handbook N.6), pp. 293-302, eds. F.T. Perkins and P.N. O'Donoghue, Laboratory Animals Ltd., London.

Phillips I.R. (1976) The embryology of the common marmoset (Callithrix jacchus). Adv. Anat. Embryol. Cell Biol. 52, 1-47.

Phillips I.R. (1976a) The reproductive potential of the common cotton-eared marmoset (Callithrix jacchus) in captivity. J. Med. Primatol. 5, 49-55.

Poswillo D.E., Hamilton W.J. & Sopher D. (1972a) The marmoset as an animal model for teratological research. Nauture 239, 272-273.

Plant T.M., James V.H.T. & Michael R.P. (1971) Conversion of (4-^{14}C) progesterone to androsterone by female rhesus monkeys (Macaca mulatta). J. Endocrinol. 51, 751-761.

Preslock J.P., Hampton S.H. & Hampton J.K. (1973) Cyclic variation of s erum progestins and immunoreactive estrogens in marmosets. Endocrinology 92, 1096-1101.

Preslock J.P. & Steinberger E. (1977a) Testicular steroidogenesis in the common marmoset, Callithrix jacchus. Biol. Reprod. 17, 289-293.

Preslock J.P. & Steinberger E. (1977b) Testicular steroidogenesis in the mature and immature baboon, Papio anubis. Gen. Comp. Endocrinol. 33, 547-553.

Rao B. (1979) Effect of prostaglandins on in vitro synthesis

of progesterone and oestradiol-17β in placenta from early human pregnancy. Prostaglandins 18, 93-99.

Rao C.H., Griffin L.P. & Carman F.R. (1977) Gonadotropin receptors in human corpora lutea of the menstrual cycle and pregnancy. Am. J. Obstet. Gynecol. 128, 146-153.

Reinius S., Fritz G.R. & Knobil E. (1973) Ultrastructure and endocrinological correlates of an early implantation site in the rhesus monkey. J. Reprod. Fertil. 32, 171-173.

Resko J.A. (1970) Androgen secretion by the fetal and neonatal rhesus monkey. Endocrinology 87, 680-687.

Resko J.A. (1974a) The relationship between fetal hormones and the differentiation of the central nervous system in primates. In, Reproductive behaviour, Advances in Behavioural Biology, Vol. 11. pp. 211-222, eds. W. Montagna, and W. Sadler. Plenum Press, New York.

Resko J.A. (1974b) Sex steroids in the circulation of the fetal and neonatal rhesus monkey: a comparison between male and female fetuses. INSERM 32, 195-204.

Resko J.A. (1977) Fetal hormones and development of the central nervous system in primates, in Regulatory Mechanisms Affecting Gonadal Hormone Action, Advances in Sex Hormone Research, Vol. 3, pp. 139-168, eds. J. Thomas and R. Singhal. H.M. and M. Medical Scientific Publishers, Aylesbury.

Resko J.A., Malley A., Begley D. & Hess D.J. (1973) Radioimmunoassay of testosterone during fetal development of the rhesus monkey. Endocrinology 93, 156-161.

Resko J.A., Ploem J.C. & Stadelman H.L. (1975) Estrogens in fetal and maternal plasma of the rhesus monkey. Endocrinology 97, 425-430.

Reyes F.I., Winter J.S.D. & Faiman C. (1973) Studies of human sexual development. I. Fetal gonadol and adrenal sex steroids. J. Clin. Endocrinol. Metab. 37, 74-78.

Reyes F.I., Boroditsky R.S., Winter J.S.D. & Faiman C. (1974) Studies on human sexual development. II. Fetal and maternal serum gonadotrophin and sex steroid concentrations. J. Clin. Endocrinol. Metab. 38, 612-617.

Reyes F.I., Winter J.S.D. & Faiman C (1976) Gonadotrophin-gonadal interrelationships in the fetus, in Diabetes and Other Endocrine Disorders during Pregnancy and in the Newborn, pp. 83-106, eds. M. New and R. Fiser, Progress in Clinical and Biological Research, Vol. 10. Alan R. Liss, Inc., New York.

Reyes F.I., Winter J.S.D., Faiman C. & Hobson W.C. (1975) Serial serum levels of gonadotrophins, prolactin and sex steroids in the non-pregnant and pregnant chimpanzee. Endocrinology 96, 1447-1455.

Rice B.F., Jacks K., Smith B. & Sternberg W.H. (1971) Steroid hormone synthesis by human fetal adrenal and gonadal tissues (Abstract) Progr. End. Soc. 53rd Meet. p.249.

Riesen J.W., Koering M.J., Meyer R.K. & Wolf R.C. (1970) Origin of ovarian venous progesterone in the rhesus monkey. Endocrinology 86, 1212-1214.

Rigg L.A. & Yen S.S.C. (1977) The pattern of increase in circulating PRL levels during human gestation. Am. J. Obstet. Gynecol. 129, 454-456.

Rivarola M.A., Forest M.G. & Migeon K.J. (1968) Testosterone, androstenedione and dehydroepiandrosterone in plasma during pregnancy and at delivery: Concentration and protein binding. J. Clin. Endocrinol. Metab. 28, 34-40.

Rolland R., Lequin R.M., Schellekens L.A. & de Jong F.H. (1975) The role of prolactin in restoration of ovarian function during the early post-partum period in the human female. I. A study during physiological lactation. Clin. Endocrinol. 4, 15-25.

Rothe H., Wolters H.-J. & Hearn J.P. (1978) eds. Biology and Behaviour of Marmosets. Proc. of the Marmoset Workshop, Gottingen, 1977. Eigenverlag Rothe, Gottingen.

Rowe P.H., Cook I.F. & Dean P.D.G. (1973) Oestrone specific antisera raised against oestrone-6-(O-carboxymethyl) oxime bovine serum albumin. Steroids Lipids Res. 4, 24-29.

Runnebaum B., Runnebaum H., Stober I. & Zander J. (1975a) Progesterone, 20α-dihydroprogesterone and 20β-dihydroprogesterone levels in different compartments from the human foeto-placental unit. Acta Endocrinol. (Copenh) 80, 558-568.

Runnebaum B., Stober I. & Zander J. (1975b) Progesterone 20α-dihydroprogesterone and 20β-dihydroprogesterone in mother and child at birth. Acta Endocrinol. (Copenh) 80, 569-576.

Runnebaum B., Holzmann K., Bierwirth A.M. & Zander J. (1972) Effect of HCG on plasma progesterone during the luteal phase of the menstrual cycle and during pregnancy. Acta Endocrinol. (Copenh) 69, 739-743.

Ryan K.J., Benirschke K. & Smith O.W. (1961) Conversion of androstenedione-4-C^{14} to estrone by the marmoset placenta. Endocrinology 69, 613-618.

Ryan K.J. & Hopper B.R. (1974) Placnetal biosynthesis and metabolism of steroid hormones in primates. Contrib.. Primatol. 3, 258-283.

Saunders F.J. & Elton R.L. (1959) Neutral steroids and maintenance of pregnancy in, Endocrinology of Reproduction, pp. 230-245, ed. C. Lloyd, Academic Press, New York.

Saxena B.B., Hasan S.H., Haour F. & Schmidt-Gollwitzer K. (1974) Radioreceptor assay of human chorionic gonadotropin detection of early pregnancy. Science 184, 793-795.

Schultz A.H. (1929) The technique of measuring the outer body of human fetuses and of primates in general. Contrib. to Embryol. 20, 213-257.

Schultz A.H. (1941) The relative size of the cranial capacity in primates. Am. J. Phys. Anthropol. 28, 273-287.

Schultz A.H. (1956) Postembryonic age changes. Primatologia 1, 887-964.

Schultz A.H. (1969) The Life of Primates, Universe Books, New York.

Schulz D.M., Giordano D.A. & Schultz D.H. (1962) Weights of organs of fetuses and infants. Arch. Pathol. 74, 80-86.

Schwarz B.E., Milewich L., Johnston J.M., Porter J.C. & MacDonald P.C. (1976) Initiation of human parturition. V. Progesterone binding substance in fetal membranes. Obstet. Gynecol. 48, 685-690.

Seppälä M., Wah lstrom T., Lehtovirta P., Lee J.N. & Leppaiouto J. (1980) Immunohistochemical demonstration of luteinizing hormone-releasing factor-like material in human syncytiotrophoblast and trophoblastic tumours. Clinical Endocrinol. (Oxf) 12, 441-451.

Serón-Ferré M., Lawrence C.C. & Jaffe R.B. (1978) Role of hCG in regulation of the fetal zone of the human fetal adrenal gland. J. Clin. Endocrinol. Metab. 46, 834-840.

Shackleton C.H. (1974) Progesterone and oestrogen metabolism in the pregnant marmoset, Callithrix jacchus. J. Steroid Biochem. 5, 597-600.

Shinada T. & Ryan K.J. (1973) The in vitro biosynthesis and metabolism of progesterone and estrogens by chimpanzee placental tissues. Steroids 21, 233-244.

Sholl S.A., Anderson, Colas A.E. & Wolf R.C. (1976) Placental and luteal steroidogenesis in the pregnant rhesus monkey. Steroids 29, 249-259.

Sholl S.A. & Wolf R.C. (1974) The metabolic clearance rate of progesterone in the pregnant rhesus monkey. Endocrinology 95, 1287-1292.

Sholl S.A., Wolf R.C. and Colas A.E. (1974) Δ^5-3β-hydroxysteroid dehydrogenase/$\Delta^5\Delta^4$-isomerase activity in the corpus luteum of the pregnant rhesus monkey. Endocrinology 94, 908-910.

Sholl S.A. Wolf R.C. and Colas A.E. (1977) Cholesterol side chain cleavage and aromatase activities in the corpus luteum of the pregnant rhesus monkey. Steroids 29, 237-248.

Short R.V. (1969) Implantation and the maternal recognition of pregnancy, in Ciba Found. Symp on Foetal Autonomy, pp. 2-26, eds. G.E.W. Wolstenholme and M. O'Connor, Churchill, London.

Short R.V. (1980) The great apes of Africa. J. Reprod. Fertil. (suppl) 28, 3-11.

Short R.V. (1974) Sexual differentiation of the brain of the sheep. INSERM 32, 121-142.

Short R.V. & Eckstein P. (1961) Oestrogen and progesterone levels in pregnant rhesus monkeys. J. Endocrinol. 22 15-22.

Shutt D.A., Smith I.D. & Shearman R.P. (1974) Oestrone, oestradiol-17β and oestriol levels in human foetal plasma during gestation and at term. J. Endocrinol. 60 333-341.

Siiteri P.K. & MacDonald P.C. (1963) The utilisation of circulating dehydroisoandrosterone sulfate for estrogen synthesis during human pregnancy. Steroids 2, 713-720.

Siiteri P.K. & MacDonald P.C. (1966) Placental estrogen biosynthesis during human pregnancy. J. Clin. Endocrinol. Metab. 26 751-761.

Siler-Khodr T.M. & Khodr G.S. (1978) Content of luteinizing hormone-releasing factor in the human placenta. Am. J. Obstet. Gynecol. 130, 216-219.

Slaunwhite W.R.Jr. & Sandberg A.A. (1959) Transcortin: A corticosteroid-binding protein of plasma. J.Clin. Invest. 38, 384-391.

Solomon S., Bird C.E., Lind W., Iwamiya M. & Young P.C.M. (1967) Formation and metabolism of steroids in the fetus

and placenta. Recent Prog. Horm. Res. 23, 297-347.

Solomon S. & Fuchs F. (1971) Progesterone and related neutral steroids. In, Endocrinology of Pregnancy, pp. 66-100, eds. F. Fuchs and A. Klopper, Harper and Row, New York.

Solomon S. & Leung K. (1972) Steroid hormones in non-human primates during pregnancy. In, The Use of Non-human Primates for Research on Problems of Human Reproduction, pp. 178-190, eds. E. Diczfalusy and C.C. Stanley. WHO Research and Training Centre on Human Reproduction. Stockholm.

Spencer R.P., Coulombe M.J. & vanWagenen G. (1966) Intraspecies comparison of fetal growth. Growth 30, 1-7.

Stabenfeldt G.H. & Hendrickx A.G. (1972) Progesterone levels in the bonnet monkey (Macaca radiata) during the menstrual cycle and pregnancy. Endocrinology 91, 614-619.

Stabenfeldt G.H. & Hendrickx A.G. (1973a) Progesterone levels in the sooty mangabey (Cercocebus atys) during the menstrual cycle, pregnancy and parturition. J. Med. Primatol. 2 1-10.

Stabenfeldt G.H. & Hendrickx A.G. (1973b) Progesterone studies in the Macaca fascicularis. Endocrinology 92, 1296-1300.

Stevens V.C., Sparks S.J. & Powell J.E. (1970) Levels of estrogens, progestogens and luteinizing hormone during the menstrual cycle of the baboon. Endocrinology 87, 658-666.

Stevenson M.F. (1976) Maintenance and breeding of the common marmoset Callithrix jacchus, with notes on hand-rearing. Int. Zoo. Yb 16, 110-116.

Stouffer R.L., Bennett L.A. Nixon W.E. & Hodgen G.D. (1977) Corpus luteum function during the early post-partum interval in lactating rhesus monkeys: *in vivo* and *in vitro* response to exogenous gonadotropin. Steroids. 29, 73-82.

Stouffer R.L., Nixon W.E. & Hodgen G.D. (1979) Disparate effects of prostaglandins on basal and gonadotropin-stimulated progesterone production by luteal cells isolated from rhesus monkeys during the menstrual cycle and pregnancy. Biol. Reprod. 20, 897-903.

Streeter G.L. (1920) Weight, sitting height, head size, foot length, menstrual age of the human embryo. Contrib. to Embryol. XI, 143-170.

Streeter G.L. (1951) Developmental horizons in human embryos. Contrib. Embryol. 34, 167-196.

Strott C.A., Sundel H. & Stahlman M.T. (1974) Maternal and fetal plasma progesterone, cortisol, testosterone and 17β-estradiol in preparturient sheep: response to fetal ACTH infusion. Endocrinology 95, 1327-1339.

Sybulski S. & Maughan G.B. (1972) Maternal plasma estradiol levels in normal and complicated pregnancies. Am. J. Obstet. Gynecol. 113, 310-315.

Talwar G.P. (1979) Human chorionic gonadotropin and ovarian and placental steroidogenesis. J. Steroid Biochem. 11, 27-34.

Tamado T., Akabori A., Konuma S. & Araki S. (1976) Lack of release of human chorionic gonadotropin by gonadotropin-releasing hormone. Endocrinol. Jpn. 23, 531-533.

Tanner J.M. (1978) Foetus into Man: Physical growth from conception to maturity. Open Books, London.

Telegdy G., Weeks J.W., Wiqvist N. & Dizfalusy E. (1970) Acetate and cholesterol metabolism in the human foeto-placental unit at midgestation. 2. Steroids synthesised and secreted by the placenta. Acta Endocrinol. (Copenh) 63, 105-111.

Thau R.B., Lanman J.R. & Brinson A. (1976) Declining plasma progesterone concentration with advancing gestation in blood from umbilical and uterine veins and fetal heart in monkeys. Biol. Reprod 14, 507-509.

Thau R.B., Lanman J.R. & Brinson A.O. (1977) Metabolic clearance rates, production rates and concentrations of progesterone in pregnant rhesus monkeys. Biol. Reprod. 16, 678-681.

Thau R.B., Lanman J.R. & Brinson (1979) Observations on progesterone production and clearance in normal pregnant and fetectomized rhesus monkeys (Macaca mulatta). J. Reprod. Fertil. 56, 37-43.

Thomas K., DeHertogh R., Pizarro M., Van Exeter C. & Ferin J. (1973) Plasma LH-HCG, 17β-estradiol, estrone, and progesterone monitoring around ovulation and subsequent

cnidation. Int. J. Fertil. 18, 65-73.

Thorburn G.D., Challis J.R. & Currie W.B. (1977) Control of parturition in domestic animals. Biol. Reprod. 16, 18-27.

Townsley J.D. (1972) Steroid hormones in pregnancy: the baboon as a model for studies on oestrogen synthesis. Acta Endocrinol. (Suppl) (Copenh) 166, 191-197.

Travis J.C. & Holmes W.N. (1974) Some physiological and behavioural changes associated with oestrus and pregnancy in the squirrel monkey. (Saimiri sciureus). J. Zool. Lond. 174, 41-66.

Treloar O.L., Wolf R.C. & Meyer R.K. (1972) The corpus luteum of the rhesus monkey during late pregnancy. Endocrinology 91, 665-668.

Tseng J.K., Adamsons K., Gaziano E., Roberts R.B. & Gurpide E. (1978) Contribution of maternal circulation to blood-borne progesterone in the fetus. 11. Studies on sheep. Am. J. Obstet. Gynecol. 130, 466-469.

Tulchinsky D. (1973) Placental secretion of unconjugated estrone, estradiol and estriol into the maternal and fetal circulation. J. Clin. Endocrinol. Metab. 36, 1079-1987.

Tulchinsky D. & Hobel C.J. (1973) Plasma hCG, estrone, estradiol, estriol, progesterone and 17α OH progesterone in human pregnancy. III. Early normal pregnancy. Am. J. Obstet. Gynecol. 117, 1095-2000.

Tulchinsky D., Hobel C.J., Yeager E. & Marshall J. (1972) Plasme estrone, estradiol, estriol, progesterone and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. Am. J. Obstet. Gynecol. 112, 1095-2000.

Tulchinsky D. & Okada D.M. (1975) Hormones in human pregnancy: IV. Plasma progesterone. Am. J. Obstet. Gynecol. 121, 293-299.

Tullner W.W. & Hertz R. (1966a) Chorionic gonadotropin levels in the rhesus monkey during early pregnancy. Endocrinology 78, 204-207.

Tullner W.W. & Hertz R. (1966b) Normal gestation and chorionic gonadotropin levels in the monkey after ovariectomy in early pregnancy. Endocrinology 78, 1076-1078.

Tullner W.W. & Hodgen G.D. (1974) Effects of fetectomy on plasma estrogens and progesterone in monkeys. (Macaca mulatta) Steroids 24, 887-897.

Turnbull A.C., Patten P.T., Flint A.P.F., Keirse M.J. Jeremy J.K. & Anderson A.M. (1974) Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before onset of labour. Lancet 1, 101-103.

Tutin C.E.G. (1980) Reproductive behaviour of wild chimpanzees in the Gombe National Park, Tanzania. J. Reprod. Fertil (Suppl) 28, 43-57.

Tyson T.E., Hwang P., Guyda H. & Friesen H.G. (1972) Studies on prolactin secretion in human pregnancy. Am. J. Obstet. Gynecol. 113, 14-20.

Vaitukaitis J.L. (1977) Human chorionic gonadotropin in, Endocrinology of Pregnancy, pp. 63-75, eds. F. Fuchs and A. Klopper, Harper and Row, London.

Vaitukaitis J.L., Braunstein G.D. & Ross G.T. (1972) A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinising hormone. Am. J. Obstet. Gynecol. 113, 751-758.

Van Wagenen G. & Catchpole H.R. (1965) Growth of the fetus and placenta of the monkey (Macaca mulatta). Am. J. Phys. Anthropol 23, 23-34.

Van Wagenen G. & Hamilton J.B. (1943) The experimental production of pseudohermaphroditism in the monkey, in Essays in Biology in Honor of Herbert M. Evans, pp. 583-607, ed. T. Cowles. University of California Press, Berkley and Los Angeles.

Vekemans M. & Robyn C. (1975) The influence of exogenous estrogen on the circadian periodicity of circulating prolactin in women. J. Clin. Endocrinol. Metab. 40, 886-889.

Vermeulen A., Verdonck L., Van der Straeten M. & Orie N. (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. J. Clin. Endocrinol. Metab. 29, 1470-1480.

Voutilainen R., Kahri A. & Salmenperä M. (1979) The effects of progesterone, pregnenolone, estriol, ACTH and hCG on steroid secretion of cultured human fetal adrenals. J. Steroid Biochem. 10, 695-700.

Walsh S.W., Meyer R.K., Wolf R.C. & Friesen H. (1977a) Corpus luteum and fetoplacental functions in monkeys hypophysectomised during late pregnancy. Endocrinology 100, 845-850.

Walsh S.W., Kittinger G.W. & Novy M.J. (1979a) Maternal peripheral concentrations of estradiol, estrone, cortisol and progesterone during late pregnancy in rhesus monkeys (Macaca mulatta) and after experimental fetal anencephaly

and fetal death. Am. J. Obstet. Gynecol. 135, 37-42.

Walsh S.W., Wolf R.C. & Meyer R.K. (1974) Progesterone, progestins and 17-hydroxypregn-4-ene-3,20-dione in the utero-ovarian, uterine and peripheral blood of the pregnant rhesus monkey. Endocrinology 95, 1704-1710.

Walsh S.W., Wolf R.C., Meyer R.K., Aubert M.J. & Frieson H.G. (1977b) Chorionic gonadotropin, chorionic somatomammotropin and prolactin in the uterine vein and peripheral plasma of pregnant rhesus monkeys. Endocrinology 100, 851-855.

Walsh S.W., Wolf R.C., Meyer R.K. & Robinson J.A. (1979b) Estrogens in the untero-ovarian, uterine, and peripheral plasma in pregnant rhesus monkeys. Biol. Reprod. 20, 606-610.

Warne G.P., Faiman C., Reyes F.I. & Winter J.S. (1977) Studies on human sexual development. V. Concentrations of testosterone, 17-hydroxyprogesterone and progesterone in human amniotic fluid throughout gestation. J. Clin. Endocrinol. Metab. 44, 934-938.

Watts E.S. (1977) Some guidelines for collection and reporting of non-human primate growth data. Lab. Anim. Sci. 27, 85-89.

Weiss G., Butler W.R., Hotchkiss J., Dierschke D.J. & Knobil E. (1976) Periparturitional serum concentrations of prolactin, the gonadotropins and the gonadal hormones in the rhesus monkey. Proc. Soc. Exp. Biol. Med. 151, 113-116.

Weiss G., Dierschke D.J., Karsch F.J., Hotchkiss J., Butler W.R. & Knobil E. (1973) The influence of lactation on

luteal function in the rhesus monkey. Endocrinology 93, 954-959.

Weiss G. & Rifkin I. (1975) Progesterone and estrogen secretion by puerperal human ovaries. Obstet. Gynecol. 46, 557-559.

Weisz J. & Ward I.L. (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses and neonatal offspring. Endocrinology 106, 306-316.

Wendt H. (1964) Erfolgreiche Zucht des Baumwollkopfchens oder Pincheaffchens Leontocebus (Oedipomidas) oedipus (Linné, 1758). In Gefangenschaft. Saugetierkdl. Mitt 12, 49-52.

Westphal U. (1971) Characteristics of steroid-protein interactions, including results on a progesterone-binding globulin. Proceedings of the Third International Congress of Hormonal Steroids. Excerpta Medica International Congress Series. 219, 410-425.

Whalen R.E., Peck C.K. & Lopiccolo J. (1966) Virilization of female rats by pre-natally administered progestin. Endocrinology 78, 965-970.

Wiedeman E., Schwartz E. & Frantz A.G. (1976) Acute and chronic estrogen effects upon serum somatomedin activity growth hormone and prolactin in man. J. Clin. Endocrinol. Metab. 42, 942-951.

Wilkins L. (1960) Masculinization of female fetus due to use of orally given progestins. J.A.M.A. 172, 1028-1032.

Wilkins L., Bongiovanni A.M., Clayton G.W., Grumbach M.M. & Van Wyk J.J. (1955) Virilizing hyperplasia: Its

treatment with cortisone and the nature of the steroid abnormality, in Ciba Foundation Colloquia on Endocrinology Vol. 8. pp. 460-481. Churchill, London.

Wilson E.A. & Jawad M.J. (1979) The effect of trophic agents on fetal ovarian steroidogenesis in organ culture. Fertil. Steril. 32, 73-79.

Wilson E.A. & Jawad M.J. (1980) LH-RH suppression of human placental progesterone production. Fertil. Steril. 33, 91-93.

Wilson E.A., Jawad M.J. & Dickson L. (1980) Suppression of human chorionic gonadotrophin by progestational steroids. Am. J. Obstet. Gynecol. 138, 708-713.

Winter J.S.D., Faiman C. & Reyes F.I. (1977) Sex steroid production by the human fetus: its role in morphogenesis and control by gonadotropins. Birth Defects 13, 41-58.

Wislocki G.B. (1929) On the placentation of primates with a consideration of the phylogeny of the placenta. Contrib. to Embryol. 20, 59-80.

Wislocki G.B. (1932) Placentation in the marmoset (Oedipomidas geoffroyi) with remarks in twinning in monkeys. Anat. Rec. 52, 381-399.

Wislocki G.B. (1938) Twinning on marmosets (Oedipomidas geoffroyi) Proc. Amer. Anat., Anat. Rec. 70,(suppl) 83.

Wislocki G.B. (1939) Observations on twinning in marmosets. Amer. J. Anat. 64, 445-483.

Wolf R.C., O'Connor R.F & Robinson J.A. (1977) Cyclic changes in plasma progestins and estrogens in squirrel monkeys. Biol. Reprod. 17, 228-231.

Wolf R.H., Harrison R.M. & Martin T.W. (1975) A review of reproductive patterns in New World monkeys. Lab. Anim. Sci. 25, 814-821.

Wolfe L.G., Ogden J.D., Deinhardt J.B., Fisher L. & Deinhardt F. (1972) Breeding andhand-rearing marmosets for viral oncogenesis studies. Beveridge Breeding Primates pp. 145-157. Karger, Basel.

Wright E.M. & Bush D.E. (1977) The reproductive cycle of the capuchin (Cebus apella). Lab. Anim. Sci. 27, 651-654.

Yannone M., McGurdy J. & Goldfien A. (1968) Plasma levels in normal pregnancy, labor and the puerperium. Am. J. Obstet. Gynecol. 101, 1058-1061.

Yoshimi T., Strott C., Marshall, J. & Lipsett M. (1969) Corpus luteum function in early pregnancy. J. Clin. Endocrinol. Metab. 29, 225-230.

Yuen B.H., Cannon W., Lewis J., Sy L. & Woolley S. (1980) A possible role for prolactin in the control of human chorionic gonadotropin and estrogen secretion by the fetoplacental unit. Am. J. Obstet. Gynecol. 136, 286-291.

Zander J., Forbes T.R., von Munstermann A. & Nehar R. (1958) Ketopregnene-20α-ol and Δ4-3-ketopregnene-20β-ol, two naturally occurring metabolites of progesterone; isolation identification, biological activity and concentration in human tissues. J. Clin. Endocrinol. Metab. 18, 337-353.

Zander J. & von Munstermann A.M. (1956) Progesteron in menschlichem Blut und Geweben: Progesteron in der placenta, in der Uterusschleimhaut und im Fruchtwasser. Klin. Wschr. 34, 944-953.